VOL. I FASC. 3

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Die Forschung über die Wirkungen und Wirkungsweisen psychotroper Substanzen hat in den letzten Jahren einen unerhörten Aufschwung genommen. Was vordem nur ein erwünschtes Ziel war, ist zu einer neuen Wissenschaft geworden: Psychopharmakologie. Da eine fruchtbare Analyse und Synthese ihrer Probleme nur durch Zusammenarbeit aller Grundfächer (Pharmakologie, Neurochemie, Neurophysiologie, Neurologie, Psychologie und Psychiatrie) möglich wird, ist die Psychopharmakologie eine verbindende, integrierende Forschungsdisziplin. Die ständig anwachsende Literatur dieses komplexen Arbeitsgebietes ist jedoch bisher zwangsläufig über zahlreiche Zeitschriften verstreut, da es bis heute kein Spezialorgan gab, das sich ausschließlich der Psychopharmakologie widmet. Diesem dringenden Bedürfnis zu begegnen, hat sich eine Gruppe von Vertretern der verschiedenen Arbeitsrichtungen der Psychopharmakologie entschlossen, eine neue Zeitschrift „Psychopharmacologia" zu gründen. In ihr sollen die bedeutenden Fortschritte dieses Arbeitsgebietes durch Veröffentlichung experimenteller und klinischer Originalarbeiten, Übersichten der neuesten Literatur sowie kurzer Originalmitteilungen zusammengefaßt werden.

Recent years have witnessed an unprecedented advance in research on the action and effects of psychotropic drugs, and what, formerly, was just a distant goal, has now evolved into a new branch of science: psychopharmacology. As, however, any fruitful analysis and synthesis of its problems can only be attained with the aid of the complete scale of basic sciences (pharmacology, neurochemistry, neurophysiology, neurology, psychology and psychiatry), psychopharmacology constitutes an integrating discipline of research. Owing to the lack of an organ devoted especially to psychopharmacology, the constantly increasing literature pertaining to this complex field of activity has hitherto of necessity been scattered among various periodicals. In order to overcome this drawback, a group of representatives of the various psychopharmacologic sections have engaged in editing a journal, "Psychopharmacologia", in which the publication of original experimental and clinical papers, reviews of recent literature and short original notices will provide a comprehensive survey of the important progress which is being actually achieved in this field of science.

Ces dernières années ont vu un développement sans précédent dans la recherche des effets et du mode d'action des substances psychotropes sur le «Comportement» et ont fait naître une nouvelle science: la Psychopharmacologie. Comme ces problèmes ne peuvent être résolus que par la collaboration des disciplines de base telles que la pharmacologie, la neurochimie, la neurophysiologie, la psychologie et la psychiatrie, la psychopharmacologie est devenue un champ de recherche de première importance. Cependant la littérature toujours croissante en ce domaine de recherche est forcément disséminée dans de nombreux périodiques, puisqu'il n'existe pas encore de journal exclusivement consacré à la psychopharmacologie. Pour répondre à ce pressant besoin un groupe de représentants des diverses disciplines de la psychopharmacologie s'est mis en devoir de rédiger un nouveau journal dans lequel seraient rassemblés les progrès importants de ce domaine, par la publication d'ouvrages originaux expérimentaux et cliniques, ainsi que des rapports sur des questions actuelles.

Richtlinien für die Mitarbeiter siehe am Schluß des Heftes. — Directions to Authors are given at the end of this number. — Directives destinées aux auteurs, voir à la fin du fascicule.

VOL. I FASC. 3

PSYCHOPHARMACOLOGIA

Contents / Inhalt / Table des Matières

Reviews · Übersichtsartikel · Revues générales

MILLER, N. E., and H. BARRY, III, Motivational Effects of Drugs: Methods Which Illustrate Some General Problems in Psychopharmacology. With 4 Figures in the Text 169

Original Investigations · Originalarbeiten · Travaux originaux

RIEHL, J.-L., J. C. PAUL and K. R. UNNA, Quantification of the EEG-Activation Response: Effects of Atropine. With 7 Figures in the Text 200

HÖHN, R., and L. LASAGNA, Effects of Aggregation and Temperature on Amphetamine Toxicity in Mice 210

JARVIK, M. E., and S. CHOROVER, Impairment by Lysergic Acid Diethylamide of Accuracy in Performance of a Delayed Alternation Test in Monkeys. With 4 Figures in the Text 221

EVANS, F. T., The Effect of Several Psychotomimetic Drugs on Human Serum Cholinesterase. With 4 Figures in the Text 231

Clinical Reports · Klinische Mitteilungen · Communications cliniques

STEVENSON, I., and T. W. RICHARDS, Prolonged Reactions to Mescaline. A Report of Two Cases. 241

Short Communications · Kurze Originalmitteilungen · Communications brèves

STEIN, L., and O. S. RAY, Brain Stimulation Reward "Thresholds" Self-Determined in Rat. With 3 Figures in the Text 251

Letters to the Editor · Briefe an die Herausgeber · Lettres à l'éditeur

BALESTRIERI, A., Studies on Cross Tolerance with LSD-25, UML-491 and JB-336. 257

Bibliographies of Current Literature · Literaturübersichten · Bibliographies

Psychopharmacology — Problems in Evaluation. Edited by JONATHAN O. COLE and RALPH W. GERARD. (MICHAEL SHEPHERD — London) 259

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Psychopharmacologia

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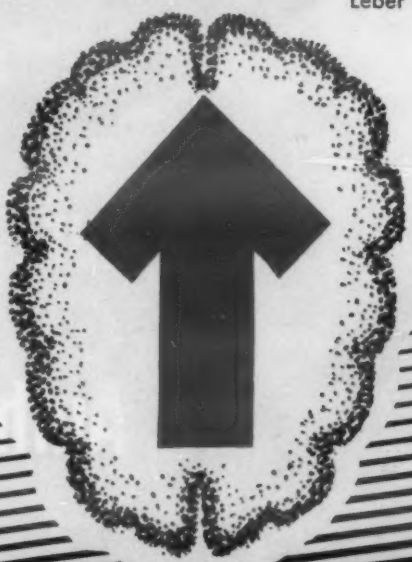

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From the Department of Psychology, Yale University, New Haven, Connecticut

Motivational Effects of Drugs: Methods Which Illustrate Some General Problems in Psychopharmacology*

By

NEAL E. MILLER and HERBERT BARRY, III

With 4 Figures in the Text

(Received October 12, 1959)

It is well known that people differ widely in their apparent sensitivity to pain and fear, in their general level of energy and activity, in fatigability, the need for sleep, and in other aspects of drive and emotion such as mood, appetite, sexuality and aggression. Furthermore, the same person may vary in these respects at different stages between infancy and senility, with changes in physical condition and disease, or in still more mysterious fluctuations. Certainly what the person has learned from his life experiences plays an important part in such differences (DOLLARD and MILLER). But it also seems that organic factors, both innate and physiologically acquired, contribute to these differences. In other words, just as hereditary and dietary factors contribute to the physical size which makes an individual a better candidate for learning varsity football, there probably are organic differences in the strength of various motivational and emotional mechanisms which make an individual a better prospect for a mental hospital. In short, the organic substrate and the conditions of social learning interact (MILLER 1957a).

At a time when it seemed relatively hopeless to do much about the organic factors, research was rightly concentrated on the environmental ones. As the recent great advances in biological techniques offer new hope that some of the organic factors contributing to the foregoing differences can be changed, the balance in research is being restored. But these new biological techniques need to be integrated with new behavioral ones to develop a basic science of the normal physiology and the pharmacology of behavior.

This paper starts with a discussion of certain techniques for the experimental study in animals of motivations and emotions[1]. In this

* Work on this paper and on studies cited in it was supported by Grants MY 2949 and M 647 from the National Institute of Mental Health, United States Public Health Service.

[1] At the Editor's request the first part of this paper recapitulates some of our previous discussions of behavioral techniques (MILLER 1950, 1956, 1957b), attempting to draw the material together and to amplify on it in the light of subsequent experience.

context, we discuss a number of the more general problems confronted in studying the effects of drugs on behavior. We emphasize the importance of fear and conflict and illustrate some new techniques with recent data from our laboratory. In the latter part of the paper we suggest certain approaches which we believe are likely to be fruitful at both the animal and the human level.

Need for a battery of tests

It will be seen that none of the tests that we shall describe are pure measures of a single variable; all of them can be influenced by a number of factors. Therefore, in using any of these tests as a measure of any one factor, such as motivation, it is necessary to hold other factors constant. But in Psychopharmacology, this is especially difficult to do since drugs are likely to have multiple effects. For this reason it is important to use a battery of tests, diversified in such a way as to control for the most probable side effects of the drug. The need for and utility of such batteries has been demonstrated with data presented in previous papers (MILLER 1956, 1957b, 1957c).

Standardized observation

There is no substitute for carefully watching the animal. The initial step in studying any drug should be to give different doses and to watch what happens in a number of simple standardized individual and group situations designed to cause the animal to exhibit a considerable range of his natural activities. NORTON has described standardized situations and check lists for observing in the cat, hamster or monkey, behavior patterns characteristic of sociability, contentment, excitement, defensive hostility and aggressive hostility. She has made statistical studies of the intercorrelations of different parts of these patterns and has shown that they are differentially affected by certain drugs.

Systematic observations of this kind, as well as the additional impressions gained by a sensitive observer, are excellent points of departure for the development of more objective behaviorial techniques. They also indicate side-effects which might contaminate the results of various objective techniques. But observation should not stop at this point; all of the apparatus for the techniques subsequently to be described should be designed with windows to facilitate sample observations. The experimenter should see exactly what the animal does and how this is affected by the drug. Such observations will help in the interpretation of the automatically recorded scores. They will also suggest ways in which the test may be refined to minimize complications from undesirable side-effects, and will be a source of new ideas.

Appetitive drives

Certain motivations such as hunger, thirst and sex may for convenience be discussed together without any assumption that they constitute a unique or unitary class.

Consummatory response. The measurement of consummatory, or goal, responses is one way to quantify appetitive drives. Thus the amount of water drunk or food eaten may be used as a measure of thirst or hunger. HILL and STELLAR have developed a drinkometer for measuring the consumption of water or liquid diets by rats; BERLYNE has used the number of novel objects investigated as a measure of curiosity and BEACH and HOLZ-TUCKER have developed methods for measuring the effects of androgen on the copulatory responses of the rat.

For certain purposes the consummatory response is the relevant measure. In developing an anti-appetite drug for the control of obesity, the most important objective is to reduce the caloric intake[1]. But the consummatory response is not necessarily always ideal. Since many animals eat or drink at a constant pace until they interrupt consumption with pauses when practically satiated, one is limited to a single measure, the total amount consumed. Then one must wait for the drive to recur. Furthermore, this single measure does not necessarily indicate the maximum intensity of the motivation, since a persistent or recurrent low level of drive which keeps the subject nibbling or sipping will induce a large amount of consumption, while an intense but more rapidly satiated drive will induce a smaller amount. Furthermore, the total consumption probably depends upon two factors: the drive to consume and the drive to stop consuming. One of the factors motivating the cessation of eating is a full stomach. Thus, at any one time, the amount consumed is artificially limited by the volume of the stomach or the ability of the body to deal with the goal substance. This factor is less limiting if the subject is given time to eat several meals.

While the consummatory response often agrees with other measures of the drive, we shall see that such agreement is by no means inevitable. For example, studies in our laboratory (MILLER 1956) have shown that although the volume of food consumed by a rat may not continue to increase with periods of deprivation longer than 24 hours, other measures do continue to increase. Similarly, MILLER et al. 1950 have shown that the bilateral lesions in the region of the ventromedial nucleus of the hypothalamus which cause a marked increase in eating, can cause a *decrease* in the rate at which animals will work for food, in the amount

[1] But a more fundamental solution would be an anti-obesity drug which changed the metabolism to that of those fortunate people who have excellent appetites, eat a lot of good food, and yet remain lean.

of quinine required to stop them from eating, and in other tests which seem to be reasonable indices of hunger.

Speed of approach. After an animal has learned to run down a short alley to a goal, its speed of running varies with drive. Since time scores have an extremely skewed distribution and give too much weight to slow performance, it is better to use either speed or log time.

BARRY, HULL, LOGAN, and MILLER and MILES (1935) have demonstrated the relationship between food deprivation in the rat and speed of running to food. SHEFFIELD et al. have shown that the running speed of male rats who copulate with the female is faster than that of non-copulating rats, and BEACH and JORDAN have shown that castration slows running speed while androgen therapy restores it. MYERS and MILLER and ZIMBARDO and MILLER have shown that, under appropriate conditions, speed of performance is a good measure of "exploratory drive".

Since running speed can be affected by factors other than motivation, one needs to cross-check it with other measures. For example, MILLER and MILES (1935) found that caffeine increased the running speed of hungry rats. This fact was probably due to the generally stimulating and disinhibiting effects of caffeine, rather than to any effects specific to hunger. The same study showed that, if rats were highly motivated and running at top speed, the drug effect was largely concealed. This is an example of the *ceiling effect*, a possibility which always must be considered in interpreting the results of drug tests. The test was much more sensitive to the effects of caffeine after the rat had been slowed down either by a decrease in hunger drive or by a series of non-rewarded trials.

The rate of operant behavior. THORNDIKE's classical problem box has been greatly simplified by SKINNER so that the animal is required to operate a single device which automatically delivers the goal object. The device is designed to be operated by a natural, relatively stereotyped movement. Rats and monkeys learn to press levers and pigeons to peck at distinctive keys. One of the great advantages of this technique is that it is so adaptable to the automatic programming and recording of long, continuous tests.

SKINNER has also introduced the extremely valuable innovation of different schedules of reinforcement. When every response is rewarded (100% schedule), most of the time is spent eating or drinking, so that the measure approaches that of a simple consummatory response. When a *fixed ratio* of responses (e.g. every 30th one) is rewarded, behavior is somewhat analogous to that of running down an alley. This schedule differentially rewards rapid behavior, so that one response becomes the cue for the next and a strongly linked chain of rapid responses is

learned. This chain is relatively stereotyped, so that the chief effect of motivation or drugs is to change the pause before the animal starts to operate the device.

When the device is set so that it only operates to deliver reward at *fixed intervals* (e.g. 60 sec. after the last reward), the animal learns to respond at a slower rate which is more susceptible to modification by drive. But he also learns a temporal discrimination: the fastest rate comes near the time when the device is about to become operative so that a response will deliver reward. When responses are reinforced on a fixed-interval schedule it seems probable that drugs can modify the rate by affecting the temporal discrimination, the drive, or in other ways such as by producing general depression, excitement, or disinhibiting the premature responses which have been extinguished.

A *variable-interval schedule* of reward eliminates the factor of temporal discrimination, and is also sensitive to changes in motivation. In studying hunger, motivated animals are first trained to press a bar which always delivers a small pellet of food into a little dish below. Then the apparatus is set so that pressing the bar will deliver food at only certain unpredictable intervals. In our experiments the rats receive on the average once every one minute a tiny pill of food weighing approximately 0.04 gm.[1] but they will work for far less rewarding schedules. Therefore, relatively long tests can be made without appreciably satiating hunger.

A large number of other schedules are possible. FERSTER and SKINNER present sample records illustrating the behavior produced by many of these schedules. In the absence of adequate systematic studies showing exactly which schedule is the most sensitive to motivation, a variable-interval schedule is a good one to use.

The general type of testing situation developed by SKINNER and his students can be adapted to a great variety of uses. In our laboratory it has been used extensively in studies of thirst, hunger, fear, "exploratory drive", "activity drive" and the aversive and rewarding effects of electrical stimulation of the brain. SCHWARTZ has used it to study sexual motivation. An ingenious study by WEISS and DANFORD illustrates the versatility of the technique. They placed shaved rats in a box at zero degrees Centigrade and taught them to press a bar to secure 10 sec. of warmth from a heat lamp. They found that the rate of working for heat was increased by dietary deficiency and further affected by pantothenic acid deprivation. There is no reason why this technique could not be adapted to study a great variety of other motivations, such as an addict's desire for drugs, the efficiency of analgesics,

[1] Purchased from P. J. Noyes Company, Lancaster, New Hampshire.

various techniques for coping with the discomfort of tropical heat, an animal's desire to rejoin the group, a gamecock's motivation to fight an opponent, and a female rat's motivation to secure nesting material or retrieve her pups[1].

Although the rate of performing an operant response has many advantages, it can be increased by general stimulating effects, or decreased by fear, distraction, or motor incoordination. It is an impure test which must be cross-checked by other different ones.

Progressive concentrations of quinine. This measure, developed in our laboratory, involves pitting the aversion produced by quinine against hunger or thirst. A series of bottle caps on the periphery of a metal disc are brought within reach of the animal, one at a time. After the animal has learned to drink from each cap as soon as it appears, he is presented at 30 sec. intervals with a series of 10 caps, each one containing 3 drops of milk (or water). After the first cap, the solution is adulterated with progressively increasing amounts of quinine sulfate: .004%, .008%, .016%, —1.0%. The animal receives two points for each cap cleaned up and one point for each cap obviously started but not completely finished. The cumulative score is found to be a sensitive and reliable measure of hunger or thirst.

Conflict induced by electric shock. The Columbia Obstruction Apparatus (JENKINS et al.) was one of the early devices for measuring drive. The animals crossed an electrified grid on the way to the goal and were said to have a stronger motivation the stronger the shocks they were willing to take. However, some animals are apparently reinforced for running forward by escaping electric shock (MILLER 1951, p. 443) and may continue to run even after they are too excited to pay attention to the goal object. This difficulty can be eliminated by giving the shock at the goal and making its termination contingent on withdrawal from the goal.

It is our impression that under most conditions the aversiveness of electric shock is more variable than that of quinine. On the other hand quinine is limited to oral ingestion, while electric shock can be pitted against the motivation for practically any type of behavior. In some cases the emotional disturbance produced by the shock may complicate the interpretation; the drugs may have a specific effect on pain or fear.

If a constant current device is used, the intensity of shock varies inversely with the area of contact; if a constant voltage source is used,

[1] For a cogent description of other ingenious uses of this versatile technique, see an article by SIDMAN (1959). This author, however, like certain other Skinnerians, believes that results by all other techniques are practically worthless, a faith which conveniently curtails the literature to be surveyed.

the shock varies inversely with the subject's resistance. When shock is administered to the feet through a grid these variations can be troublesome, but a simple compromise is reasonably effective. Current from a step-up transformer is controlled by a voltage divider and applied through a series resistance of 200,000 Ω. In some cases we have controlled intensity by permanently implanted electrodes in the scalp or flank. The problem of a grid being shorted by the rat's feces is controlled by making the bars 5/8 in. in diameter and spacing them 1—1/8 in. between centers (DINSMOOR) and by using a grid scrambler circuit which energizes different sets of bars at different times. For alleys we use smaller bars or the system described by CAMPBELL and KRAELING.

Strength of pull. Another measure, developed and used in our laboratory, is the strength with which an animal will pull when temporarily restrained on the way to the goal (BROWN). This measure has been used in studies of the effects of alcohol (CONGER) and sodium amytal (BAILEY and MILLER). To date we have not attempted to repeat this measure many times on the same subject. It is conceivable that repetitions would be practicable if one incorporated the feature of a variable-interval schedule of release. Obviously, this measure will be affected by muscular strength; in fact, it might be at least as useful for measuring strength or fatigue as motivation.

Preference tests. In this technique, the animal is given a choice between two or more goal objects. This method has been useful in studies of specific hungers and palatability which have been summarized thoroughly by YOUNG. The preference test yields a relative measure which will be affected by changes in the preference for either of the contrasted goal substances. It should be noted that the most preferred substance is not necessarily the one that will elicit the largest volume of consumption.

Importance of fear and conflict

Studies of men under the intense stress of combat show that great fear, and/or the conflict it induces, can elicit in previously normal soldiers the gamut of symptoms observed in neuroses and psychoses (MILLER 1951).

It is well known that these reactions to intense fear and conflict can be consolidated into relatively permanent combat neuroses. Similarly, in civilian neurotic and psychotic behavior, anxiety and the conflict it generates seem to play an important role. And even within the normal range, fear contributes to the tensions and fatigue of everyday life. In the light of such evidence, we believe it is especially important to study systematically the effects of drugs on both acute and chronic fear and conflict.

Techniques for measuring fear

Standardized observation. The methods of standardized observation to which we have already referred can be used to measure fear. In the rat some of the common symptoms useful on a check list are remaining motionless, avoiding open spaces, elevation of the tail, urination, defecation and unsheathing of the claws. In other animals one can frequently observe dilation of the pupils and bushing of the hair, especially on the tail. Again it is important to observe the animal even in an automated test apparatus in order to be able to interpret the results and devise new measures.

Action threshold. For measuring the level at which a gradually increasing stimulation, such as electric shock or direct stimulation of the brain, becomes disturbing enough to the animal so that he is motivated to do something about it, a stepping relay wired as a voltage divider increases the level of stimulation by stepping up once every 1 sec. This stimulation can be turned off by a response, such as rotating a wheel or pressing a bar. Then after a variable interval of time, the stimulus starts increasing again.

Rats will rapidly learn to escape electric shock in this situation and will settle down to a relatively constant pattern of responding. They have a moderate probability of responding immediately after the shock has been turned off, and then a relatively low probability of response until the threshold is presumably reached, at which point the probability of responding increases rapidly. Thus some responses are conditioned anticipatory responses to fear; others are unconditioned escape responses to pain plus fear of shock. As might be expected, we have found that the behavior is considerably less variable when, instead of an electric shock to the feet through a grid floor, the stimulation is given through fixed electrodes under the skin of the scalp or through electrodes implanted in aversive areas in the brain. But to our surprise, the effect of the drugs we have tried to date is also reduced when fixed electrodes are substituted for the grid floor. We are not yet certain whether this reduction in drug effect completely cancels out the reduction in error variance.

Avoidance conditioning. The animal is presented with a cue which is followed after say 5 sec. by electric shock. If he responds promptly, he terminates the cue and avoids the shock. If he does not respond promptly, he receives the shock. In the latter case, the only way that he can turn off the shock is usually, but not necessarily, to perform the same response that would have avoided it. A great variety of responses have been used in avoidance conditioning: running in an activity wheel, shuttling from one compartment to another, rotating a squirrel-cage or paddle wheel projecting into the apparatus or press-

ing a bar or a panel. The measures are percentage of successful avoidance responses and/or speed of response (MILLER 1951, SOLOMON and BRUSH).

When the cue is that of being dropped onto the grid of a white compartment and the response is jumping over a little hurdle into a black compartment with a smooth floor, we have always secured rapid avoidance learning with rats. Under other conditions, avoidance learning may be more difficult than one might gather from published reports. Animals being trained to shuttle back and forth from one compartment to another whenever a buzzer is sounded may develop conflict about re-entering the compartment where they previously were shocked. Animals given long sessions every day may acquire and then unaccountably lose the avoidance habit.

MYERS has shown that if a strong stimulus, such as a buzzer, is used as a cue for turning a wheel to avoid shock, it will elicit practically as much responding in a group given pseudo-conditioning trials, in which the cue and shock are presented separately and never paired, as it will in the group for which the buzzer is the signal for the shock. Thus the responses measured may well be pseudo-conditioned, potentiated startle responses rather than genuine avoidance responses. A weaker stimulus, such as a moderate tone, does not elicit pseudo-conditioned responses, but may produce a very low level of avoidance-conditioning. This state of affairs is greatly improved if the shock is also put on the wheel (or bar) by including it in the grid-scrambler circuit.

Certain other precautions should be observed. The level of intertrial responding may be fairly high. Unless one records such responses, one cannot be sure that the responses to the danger cue are more frequent than those that would have occurred during the same period of time. One should select simple responses, such as pressing a low bar, that are least affected by the incipient motor incoordination or paralysis which seems to affect first the ability to stand up on the hind legs. Similarly, one should use at least two different modalities of cues (perhaps alternating them for efficiency) in order to have some check on specific sensory deficits.

The *strength-of-pull* technique (BROWN) can be used with avoidance conditioning. One measures the force with which a temporarily restrained animal pulls in attempting to run from a dangerous to a safe place.

We are starting to explore an *avoidance threshold* technique, by using a signal, such as a tone, that gradually increases in intensity. When the tone reaches a sufficiently high intensity, an electric shock will be given — alternatively, at unpredictable times, electric shocks may

infrequently occur, being weak when the tone is weak and strong when the tone is loud. Pressing a bar will reset the tone to zero, or alternatively, might step it down to the next lower intensity.

Finally, SIDMAN (1953) has devised an ingenious technique in which pressing a bar postpones the occurrence of the shock for a specified interval. The conditioned stimulus in this technique seems to be associated with the passage of time. It is not easy to train rats in this response, but they will keep on performing it for long periods of time.

Comparing conditioned avoidance with unconditioned escape. A drug often will eliminate a conditioned avoidance response, presumably motivated by fear, before it will affect the unconditioned response to the pain of electric shock. Such a result could mean (a) that the weaker response was affected first, or (b) that the drug affects a response that is motivated by fear more than it does one motivated by pain. Before concluding that there is a differential effect on fear it is necessary to equate the strengths of the two drives, comparing a moderately strong fear (based an stronger pain) with a moderately strong pain. The type of comparison required is illustrated by MILLER and MURRAY.

Potentiated startle. D. R. WILLIAMS[1] has ingeniously developed a technique for automatically programming and recording the potentiating effects of fear on the startle response, which were reported originally by BROWN et al. A rat is confined in a small light-weight box with a grid as a floor. The box is suspended from a pulley connected to a spring in such a way that the movements of the animal will cause the teeth on a large light-weight pasteboard wheel to interrupt the beam to a photocell. Relays are programmed to switch the output of the photocell to a printing counter which records the number of beam interruptions during the 0.75 sec. interval when the rat is making a reflex startle to a given stimulus. Every 90 sec. a flashing light comes on for 4.5 sec. being terminated by a momentary electric shock. In addition, a loud beep is presented at various times. From the solid line in Fig. 1 it can be seen that the startle response to this beep is smallest when it is presented between trials and becomes progressively larger when it is presented during the flashing light nearer to the time at which the shock would normally be delivered. The response to the beep is greatest on those trials when the shock is omitted and the beep is presented at exactly the time when the shock would normally occur. It can also be seen that different doses of alcohol have relatively little effect on the startle to the beep between trials but do reduce the potentiation of the startle by the fear-evoking CS of flashing light.

[1] Throughout this paper we use the initials of the investigator along with his name in crediting work from our laboratory which has not yet been published.

Approach-avoidance conflict. An animal is trained to perform a response, then an incompatible response is elicited by giving him an electric shock or other noxious stimulus. For example, a hungry animal may be trained to run down an alley for food and then given increasingly strong shocks at the goal. Measures can be taken of the speed of running, the strength of shock necessary to deter him from reaching the goal, and the distance which he approaches toward the goal after deterrence. This technique has been used with alcohol by CONGER and sodium amytal by BAILEY and MILLER.

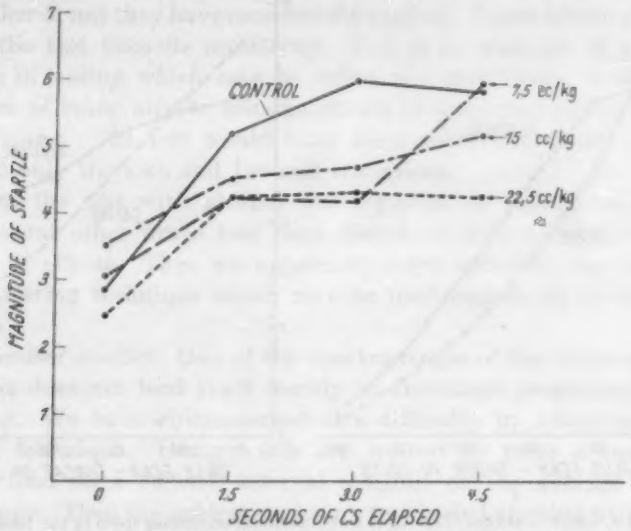

Fig. 1. Effect of alcohol on potentiated startle. Doses are in cc/kg of a 10% solution injected intraperitoneally. The zero sec. of CS elapsed is a presentation of the startle stimulus given between trials, the 4.5 sec. is one presented at the time the shock would normally occur

The conflict technique is probably less sensitive to motor side-effects than the various avoidance techniques but is influenced by effects of the drug on the approach as well as on the avoidance drive used. This dual effect is a disadvantage in measuring absolute effects, but an advantage in measuring relative ones, since the other drive serves as an automatic control. Using a number of different approach drives is one way to determine the specificity of the drug effect to the drive motivating the avoidance.

In previous uses of the conflict technique (MILLER 1959), the tests have not been repeated many times on the same animal nor have they been readily adaptable to automation. Recent work in our laboratory has overcome these limitations.

Telescope alley. We have trained rats to run down an alley with the end plate containing the food dish designed so that it could be in-

serted at various one-foot distances from the start to the full length of six feet. After having learned to run different distances, each rat was given a series of immediately successive pairs of trials of running progressively greater distances, with no shock for running one foot on the first two trials, and progressively stronger shocks for running the longer distances on the succeeding pairs of trials. After approximately 8 days, each animal reached a stable level of performance, running fast

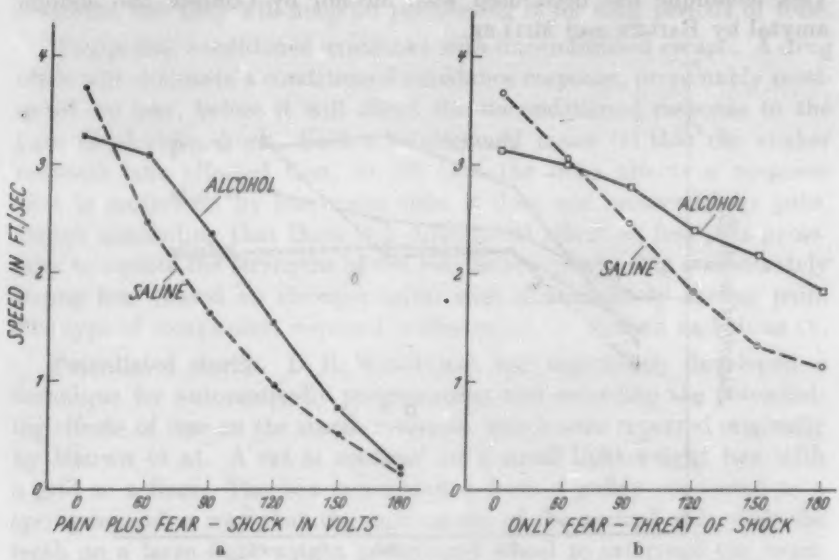

Fig. 2 a and b. Effect of alcohol (15 cc/kg of a 10% solution) on running speed in the telescope alley. Since the average starting time was found to be equal to that of running approximately 3 feet, it was necessary to calculate speed in ft. per sec. by the formula 1/Time X (Distance in feet + 3) in order to secure comparable scores for the different distances

on the short safe trials, slower on the longer more dangerous ones, and finally quitting as the shock became too strong. The test-retest reliability was high —.96.

After further training during which the occurrence of the shocks was unpredictable, but the strength of those that occurred was correlated with the length of the alley, the rats continued to slow down progressively and stop at the longer distances during a test sequence with a shock only at the end of the longest run, or in other words, after the test was completed. We used such fear tests (without shock) as controls for any analgesic effects of the drug. We also gave other tests with unpredictable shocks on the shorter runs, in order to prevent the rats from learning that only the longest distance was dangerous.

Fig. 2 shows the effect of alcohol (15 cc/kg of a 10% solution), injected intraperitoneally. The left-hand side of the figure shows the

effects when the appropriate level of shock is given at each length of the alley; the right-hand side shows the results when shock is given only at the end of the last and longest run. In both cases (a) the alcohol decreases the speed of running the short safe distance, (b) the rats run progressively slower for the increasingly dangerous distances, and (c) the alcohol reduces the decrement so that the drug and control curves cross. Comparing the two sides of the figure shows that giving shocks throughout the test, slows the animals down more; in fact, at the longest distances most of them fail to reach the goal, irrespective of whether or not they have received the alcohol. Thus with the strongest shock, the test loses its sensitivity. This is an example of a general problem in testing which may be called the *floor effect.* It illustrates the value of being able to test the effects of drugs throughout a range of conditions. The test would have been much less useful if it had included only the zero and 180 volt conditions.

When the test with alcohol was repeated 30 testing days later, after several other drugs had been tested, it still yielded the same pattern of effects. Thus we apparently have achieved our goal of a conflict-testing technique which may be used repeatedly on the same subjects.

Automated conflict. One of the disadvantages of the telescope alley is that it does not lend itself readily to automatic programming and recording. We have circumvented this difficulty by adapting a bar-pressing technique. Hungry rats are trained to press a bar which delivers food on a variable-interval schedule on the average of once every 1 min. Then the subject is given a test period starting with 4 min. of silence, after which a tone starts out at a low level and is increased in loudness every 10 sec. to a maximum level at the end of 4 min. of tone, and then drops to zero for the final 2 min. While the tone is on, a 1 sec. shock is given via the grid floor at unpredictable times which occur on the average of once every 75 sec. Because both the shock and the tone are controlled by separate banks of a stepping relay wired up as a voltage divider, the intensity of the shock, when it occurs, is perfectly correlated with the intensity of the tone. The rats can avoid the shock by retreating to a plastic strip which covers the grid on the side of the enclosure away from the bar. (In an alternative procedure which may be superior, we have administered the shock from the bar to the grid.)

In order to control for possible analgesic effects of the drug, we usually give two test sessions. In the first of these the shock occurs only at the end of the tone. Hence the preceding behavior is affected only by fear and not by pain. In the second test session, the shock occurs at unpredictable intervals any time during the tone. This procedure

prevents the animals from forming a discrimination that they are safe until the loudest level of tone.

Fig. 3 shows a graph of 6 rats tested in a balanced order after injections of either isotonic saline, or 20 mg/kg of sodium amytal. These were fear tests with the shock occurring only at the end of the tone. The graph shows that the rate of bar-pressing decreases as the tone becomes louder and the animal runs the risk of getting a stronger shock. Before the tone begins, the rate is lower during the sodium amytal test than during the control one. But the fear elicited by the increasingly loud tone produces a much greater decrement in the control test than it does in the experimental one, so that the curves cross and the drugged rats are performing reliably better.

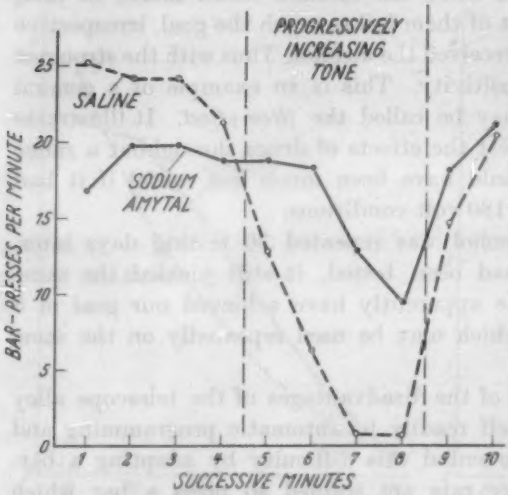

Fig. 3. Effect of sodium amytal (20 mg/kg) on performance of automated conflict. The first 4 and last 2 minutes are without tone; during minutes 5—8 the tone progressively increases in intersity, a condition which in the past (but not on this test trial) has been correlated with the unpredictable occurrence of increasingly strong electric shocks which could be avoided by leaving the bar

Conditioned suppression (CER). ESTES and SKINNER have exploited the tendency of fear to produce freezing, which depresses the rate of pressing a bar to secure water or food. Studies with this technique have been summarized by SIDMAN (1959). A stimulus, such as a clicking sound, is presented at various times and terminated by electric shock. After a number of trials, the animals stop bar-pressing during the clicking sound. After an enormous number of trials they may stop only immediately before the shock. In an attempt to avoid this defect and to give the measure a wider range, varying from moderate to complete suppression, we have modified this technique by using a stimulus which gradually increases in intensity and is correlated with the intensity of the shock. Our procedure is exactly like that which has just been described for automated conflict, except that the safety island is removed so that the electric shock can neither be escaped nor avoided and is not specifically associated with performing the response to be inhibited. As with the conflict technique, the relative strengths of two drives are involved. In both cases we plan to try danger signals in other

modalities in order to control for effects of drugs which are specific to a given modality.

Physiological measures. LINDSLEY has described the use as indices of emotional disturbances of a number of physiological reactions, such as galvanic skin response, blood pressure, heart rate and skin temperature. LACEY and WENGER believe that the patterns of such reactions are much more useful than the individual reactions, but disagree on exactly how these patterns should be interpreted. With such measures, a direct drug effect specific to the physiological reaction can be a source of error much as the drug may have an effect on some aspect of the behavior sequence other than the motivational one.

LILLY has described the step-by-step development by a number of investigators of a monkey-restraining chair for physiological and psychological research. MASON and BRADY have used this chair to record simultaneously avoidance behavior and changes in the blood levels of substances such as the corticosteroids. Drugs are injected or blood samples are withdrawn through a chronic catheter into a jugular vein. The monkey can be kept for many days in the chair which allows him freedom to perform behavioral tasks but prevents him from pulling out the chronic catheter in the vein or electrodes in his brain.

A specific drive to escape conflict. PAVLOV and GANTT have shown that gradually working an animal up to a discrimination that is too difficult for him can produce a striking emotional disturbance, a so-called "experimental neurosis", with a variety of rather widespread effects. SAWREY, CONGER and TURRELL present evidence indicating that the conflict produced by giving hungry animals electric shocks when they go to food or water has a more damaging effect, as measured by the ulcers produced, than merely exposing the animals to an equal amount of food and water deprivation and electric shock without inducing a specific conflict about getting food and water. Clinical observations, especially of war neuroses, also suggest that intense conflict can be involved in producing a variety of neurotic and even psychotic-like symptoms (DOLLARD and MILLER). For these reasons it seems especially important to study the effects of drugs on conflict.

Observations by PAVLOV and GANTT show that the animals seem to hate being brought into the experimental-neurosis situation for training. But other animals show considerable distaste for the monotonous confinement in the conditioning stand, even without such training. Perhaps the desire to escape is not really produced by the conflict, but only becomes more evident when the animal has less reason to tolerate the confining stand.

MILLER (1959, pp. 236 and 237) has suggested a way to determine whether a drive specifically to avoid conflict is induced and how to

study any such drive. Essentially the animal remains in the apparatus but is given the opportunity to turn off the stimulus which signals the conflict trials and thus to expose himself to a changed stimulus which signals time out from the conflict problem. It is as if by pressing a bar he could cause the discrimination apparatus to fall through the floor. Then one should be able to measure the motivation to perform such a response in the same way that one can measure the motivation to work for food. Such a measure would be useful as an index of the disturbance specifically caused by conflict. If a drug can be found which will make it easier for a subject to tolerate working in a conflict situation — in other words reduce the conflict-induced drive without interfering with the other motivations — it might be an ideal therapeutic agent.

At present we are working on this problem using two types of situation: (a) a difficult discrimination which does not involve punishment by shock or any other painful stimuli, and (b) an approach-avoidance conflict situation. While it is too early to announce any definitive results, our work indicates that an animal will press a bar to avoid exposure to "temptation" in an approach-avoidance conflict, and that a drug can affect the rate of pressing this bar differently from the rate of pressing the conflict bar for food and shock. HEARST and SIDMAN are also securing similar evidence for motivation to escape from an approach-avoidance conflict. We believe that further work along this line is especially likely to be profitable.

Need for analytical studies — social effects of alcohol

We have described a number of different behavioral techniques which are useful in studying the normal physiology of motivation and the effects of drugs. We believe that one of the most fruitful uses of these techniques is to study analytically how specific drugs affect motivation. Such studies are needed for the healthy development of the infant science of Psychopharmacology. It should be profitable to start with familiar drugs known to have significant effects. The type of work needed may be illustrated by some experiments on the social effects of alcohol.

The social effects of alcohol are puzzling. Although this drug supposedly is a depressant, the increase in noise level after drinks at a party does not obviously indicate depression. One assumption is that the higher functions are depressed first, releasing the lower ones. But it is not clear that standing shyly and mutely in a corner necessarily involves functions that are higher than those of becoming the life of the party. Furthermore, alcohol has a perplexing variety of effects,

making some aggressive, others amorous, some tearful, and others talkative. Certain people are pleasantly released while others are relatively unaffected or even made unpleasantly tense.

An early study of alcohol by MILLER and MILES (1936) started with the hypothesis that Freudian repression and Pavlovian inhibition are related. Both prevent the occurrence of a response but can be demonstrated not to remove the response tendency. There are a number of other interesting parallels. If these two phenomena are related, and if the alcohol tends to weaken Freudian re- pressions, it should also tend to reduce Pavlovian inhibitions. In other words, it should have a disinhibiting effect on an experimentally extinguished response. This hypothesis was tested by training albino rats to run a simple maze, then partially extinguishing their response by a series of non-rewarded trials. If alcohol has a disinhibiting effect, or if it is more disrupting to more recent learning, we would expect it to cause these "discouraged" animals to run faster. As Fig. 4 shows, the results were exactly the opposite. The alcohol, which slowed down the rewarded rats, likewise slowed down rather than speeded up the partially extinguished ones.

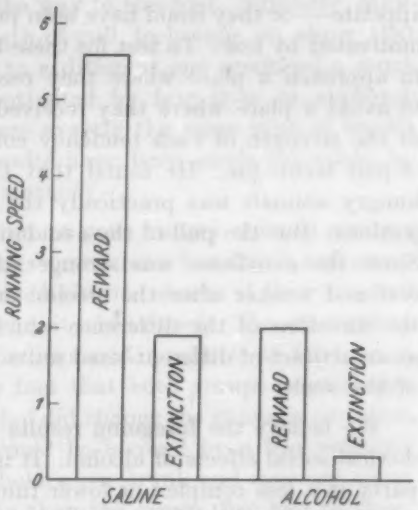

Fig. 4. The effect of alcohol (10 cc/kg of a 10% solution) on running speed in a simple maze, under conditions of reward and of experimental extinction. Data from MILLER and MILES (1936)

A number of years later MASSERMUM and YUM did an experiment which caused them to conclude that alcohol differentially disrupted the more complex and recently acquired "neurotic" behavior of their cats. In our laboratory, CONGER thought that there might be a simpler explanation, namely, that alcohol differentially reduces the avoidance component of the simple approach-avoidance conflict. He tested this by training hungry rats to run down a short alley to get food. After they had thoroughly learned, he gave them increasingly strong electric shocks at the goal until they just barely stopped eating there. After a control injection of isotonic saline, the rats still refused to run to the goal and eat; after a moderate injection of alcohol, they resumed running to the goal and eating there.

Our results in the telescope alley (see Fig. 2) confirm this finding in a situation in which both habits were relatively old, whereas in CONGER'S experiment the avoidance responses were much more recent. These indicate that recency was not the main factor in making the avoidance habits more susceptible to alcohol.

These results could have been produced by an increase in approach motivated by hunger — the cocktail before dinner increasing their appetite — or they could have been produced by a decrease in avoidance motivated by fear. To test for these factors, CONGER trained one group to approach a place where they received food and a different group to avoid a place where they received shock. The rats wore harnesses, so the strength of each tendency could be measured by the strength-of-pull technique. He found that the pull of temporarily restrained hungry animals was practically the same after control or alcohol injections. But the pull of the avoidance was greatly reduced by alcohol. Since the avoidance was stronger than the approach on the control test and weaker after the alcohol one, he demonstrated a reversal in the direction of the difference which could not possibly be explained as an artifact of different-sized units of measurement at different points of the scale.

We believe the foregoing results help to explain some of the paradoxical social effects of alcohol. It is not that becoming the life of the party is a less complex or lower function than standing mutely in the corner, but rather that the lively social behavior has been inhibited by conflict and is released when alcohol reduces the avoidance motivated by fear. A person with barely inhibited sexual tendencies will become amorous; one with barely inhibited hostile tendencies will become aggressive. Finally, as DOLLARD and MILLER have explained in detail, the effects should vary with the relative strength of the tendencies in conflict. People with strong repressions may be shifted from the comfortable position of not being tempted at all, to the uncomfortable one of being released enough to be strongly tempted while in too much conflict to achieve their goal.

In these studies the behavioral techniques contributed to our understanding of the effect of a drug, alcohol; but the drug also contributed to our understanding of behavior. It showed that the inhibition of a habit by non-rewarded trials is different from the inhibition of a habit by a conflicting response. These two phenomena must be different since the alcohol intensifies the effects of one, but alleviates those of the other. The interaction between experimental psychology and the study of drugs is a two-way affair; Psychopharmacology can make contributions to a basic science of behavior.

Need to control for stimulus change. The alcohol should change the stimulus context for the rats by introducing a variety of novel sensations, such as tingling and staggering. But it is known that changes in the stimulus situation can affect avoidance more than approach. MILLER and KRAELING found that, if hungry rats were trained to run down a narrow black alley for food and then given shocks at the goal until they stopped running, shifting them to a wide white alley would cause them to resume running all the way to the goal. Similarly, MURRAY and MILLER used the strength-of-pull technique to show that changing rats trained in one alley to a different one produced a much greater reduction in avoidance motivated by fear than in approach motivated by hunger. But these are exactly the same type of results secured by CONGER. Could his results have been produced solely by the stimulus changes induced by alcohol?

To control for this possibility CONGER gave hungry animals running down an alley for food a series of trials, sometimes when drunk and sometimes when sober. One group received electric shocks if they touched the food when sober, but not if they ate it when drunk. The other group was trained under the opposite conditions of no-shock at food when sober but shock when drunk. The fact that both groups learned this discrimination showed that the alcohol did change the stimulus situation for the rat. But the group that would be favored by a fear-reducing effect of alcohol — learning to be bold when drunk and cautious when sober — learned much more rapidly than the group that had to overcome this fear reducing-effect and learn to be careful when drunk and bold when sober.

Another way to control for the stimulus changes induced by a drug is illustrated by S. P. GROSSMAN's experiment in our laboratory. He used a 2×2 factorial design for studying the effects of alcohol in the telescope alley. Half of the animals received their original avoidance training with electric shock when drunk and the other half received it when sober. Then each group was split into two halves, one of which received tests when drunk and the other when sober.

The design and experimental results are illustrated in Table 1. Adding up the rows shows the effects on learning of having the drug during previous training; the difference is small and not statistically significant. Adding the columns shows the effects on performance of having the drug during testing; the difference is large and statistically significant. Comparing the diagonals shows that the changed groups are similar to the unchanged ones. In other words, there is little or none of the interaction that might be expected if the drug altered the stimulus situation. In each of these comparisons other effects are

balanced out. Thus GROSSMAN confirmed CONGER's conclusion that alcohol effects cannot be explained solely by stimulus change.

In addition to illustrating a type of analytical study that is desirable, our discussion of alcohol has indicated four types of general factors which should be, but often are not, controlled in the studies of the effects of drugs:

(a) Control for the general depressing effect of the drug. This was achieved by testing the effect of alcohol on the strength-of-pull for hunger-motivated approach as well as for fear-motivated avoidance.

Table 1. *Design and results of experiment controlling for stimulus change as a possible source of the effect of alcohol in the telescope alley.*

Scores in the boxes are the average number of trials to deterrence in a test without shocks. Based on unpublished data by S. P. GROSSMAN.

Training \ Testing	Drug	Control	Sum
Drug	D—D 5.5	D—C 4.3	9.8
Control	C—D 6.0	C—C 2.8	8.8
Sum	11.5	7.1	18.6

(b) Control for the possibility that the measurement may be in units which are not psychologically equal at different points of the scale. This was achieved by selecting values so that the two curves crossed. The precautions required by the fact that behavioral tests often do not yield equal-interval scales are discussed further by MILLER (1959, pp. 281—286).

(c) A control for drug-induced stimulus changes. This was achieved by using a 2×2 design.

(d) Control for the possible analgesic effects of alcohol by tests with fear but without pain.

Usefulness and limitations of fear-reducing drugs

As DOLLARD and MILLER (Chapter 23) point out, the person who takes alcohol to achieve the socially desirable effect of being less shy at a party may also achieve the undesirable effect of driving recklessly on the way home. One cannot expect a drug to separate the socially undesirable fears from the desirable ones. Thus the goal is not the permanent and complete abolition of all fear. With proper dosage a suitable drug might reduce a person with a generally hyperactive fear mechanism to a more normal level. But the medication would have to be continued, unless interventions of the type we shall discuss under developmental psychopharmacology should be possible.

Many of the fears encountered in the clinic are relatively specific — the student who is terrified at presenting a report in class may be fear-

less on the football field. These fears must be dealt with by the process of re-education, teaching the patient to make discriminations between socially appropriate and socially inappropriate fears. But even here, as DOLLARD and MILLER point out, drugs may be of some use. Temporary administration of the drug in the appropriate situation may reduce the level of fear enough to help the patient to get started on the process of relearning. A more effective fear-reducing drug could be a great aid to psychotherapy.

Although one of the most important potential uses of fear-reducing drugs is to facilitate socially desirable learning which has been inhibited by fear, relatively little experimental work has been done on this problem. As DOLLARD and MILLER note, one of the crucial points to investigate is the generalization from the drugged to the normal state. A study by HUNT on chlorpromazine has failed to find evidence for such generalization; one by BLACK on a curare-type drug has shown generalization of the therapeutic effect. Since we are not certain that all drugs which seem to hasten the extinction of fear will produce an effect which generalizes to the non-drugged state, specific tests for such generalization should be made.

Systematic comparison of measures — do drugs affect fear as an unitary variable?

We also need analytical studies to learn more about the properties of our measures and the effects we are trying to measure. This type of work is illustrated by a current study in our laboratory. We are trying to compare systematically the effects of a number of well-known drugs, purporting to affect fear, on a number of behavioral techniques purporting to measure fear. Do all of these techniques measure the same thing, and if so, are some considerably more reliable and sensitive than others? Are some techniques more susceptible than others to certain side-effects of the drugs? Does fear function as a single unitary variable or is it a constellation of variables, some of which may be affected by certain drugs and others by different drugs? For example, do certain drugs primarily affect the pattern of freezing and being mute, while others primarily affect the pattern of running and squealing?

In this study we are using on rats the types of techniques already described as measures of fear and conflict. But in order to see whether the effects are specific to fear, we plan to use also other techniques. For example, we plan to slow down the behavior in other ways, such as by experimental extinction, differentially rewarding slow behavior, or rewarding motionless behavior (BLOUGH), techniques which may also involve a considerable amount of conflict.

We have already progressed far enough to see that the results probably will not present a completely simple picture. For example, chlorpromazine seems to have a marked "fear-reducing" effect on the automated conflict and conditioned suppression techniques, and relatively little, if any, clear-cut effect in the telescope alley. By contrast, alcohol seems to have a marked effect in the telescope alley conflict and little or none in the automated conflict, but probably some in the conditioned suppression. Sodium amytal seems to have marked fear-reducing effects on all of these techniques. Other examples give additional evidence for situational-drug interactions. We plan to repeat these tests to make sure that the anomalies are not statistical artifacts and then to try to devise reasonable hypotheses to explain these results and to design experiments to test these hypotheses.

After we get a better understanding of the tests and the variables they measure, we plan to repeat a selected battery on monkeys in order to get some idea of the effect of the phylogenetic variable. It is true that both rats and people press a bar rapidly if they are rewarded for rapid performance and slowly if they are rewarded for slow performance. But we do not agree that the fact that certain behavioral principles are the same at different phylogenetic levels should be interpreted to mean that the effects of all drugs on the behavior of different mammals necessarily will always be the same.

Finally, we hope to find out whether the effects of drugs are the same on equally intense fears based on different unconditioned stimuli, such as pain or the presentation of a snake to a chimpanzee.

Combined behavioral-physiological analysis

It is obvious that a better understanding of the physiology, biophysics, and biochemistry of behavior will play a basic role in psychopharmacology. Ultimately we want to know much more about the behavioral effects of specific metabolites and anti-metabolites, enzymes and enzyme inhibitors, hormones and anti-hormones and the factors that govern storage and release from specific binding sites in the brain.

At a vastly simpler level, a series of studies of hunger which have been described elsewhere (MILLER 1957c) illustrates some halting first steps in the direction of combining physiological and behavioral techniques. We have found that food injected directly into the stomach produces an immediate reduction in hunger, while food ingested normally by mouth produces an even greater reduction in hunger. Distension of the stomach by food injected directly by chronic fistula serves as a reward to cause animals to learn to choose the arm of a T-maze where the injection is given, but distension of the stomach by the inflation

of a balloon on the end of a similar fistula serves as a punishment to cause animals to avoid that arm of the T-maze. Electrical stimulation of certain areas of the hypothalamus not only induces eating of a variety of solid and liquid foods, but also causes animals to work at responses they have learned as means of getting food. Finally, E. E. Coons has found that intraperitoneal injections of d-amphetamine appreciably raise the threshold of eating elicited directly by electrical stimulation of this area in the brain.

The next logical steps in this program involve trying to determine whether we are dealing with a brain center or only a pathway. One test will be to see whether eating can be elicited by minute injections of acetylcholine. We also hope to test the effects of minute injections of d-amphetamine directly into this area[1].

If negative results, especially with acetylcholine, indicate that we have not located a "hunger center", we shall have to search elsewhere. With positive results, it will seem worthwhile to try injections of other chemicals, including extracts from the blood of hungry or supersatiated animals. Whereas A. A. Campbell's work with parabiotic rats (artificial, surgically created "siamese twins") in our laboratory has not succeeded in securing any evidence for a hunger hormone or indeed for the transfer of appreciable amounts of nutrients across the parabiotic barrier, Hervey, in England, has shown that if one member of a parabiotic pair is made hyperphagic by hypothalamic lesions, the other member of the pair tends to waste away. This suggests that when the target brain center is damaged, an abnormal amount of some sort of satiation substance accumulates before the hyperphagic rat finally stops eating. If the injection of minute amounts of blood from a satiated hyperphagic rat into appropriate sites in the brain arrests normal hunger, we shall have a preparation which may allow us to begin the tedious process of fractionating the blood to try to isolate the satiation substance or substances. Such a substance, or some similar compound, might be a powerful anti-appetite drug.

While this particular line of work may not pay off at this time, we believe it illustrates a desirable combination of behavioral and physiological techniques in a series of analytical studies.

Developmental psychopharmacology

A deficiency in a simple chemical, namely iodine, during infancy causes cretinism characterized by striking and lasting intellectual

[1] Since this was written, S. P. Grossman has found in our laboratory that tiny amounts of crystalline epinephrine introduced into this general area can elicit vigorous and prolonged eating and drinking by satiated rats. Apparently other substances, such as acetylcholine and strychnine, do not necessarily produce such a striking effect when introduced via the same chronic cannula.

deficits. Supplying iodine during the appropriate years is therapeutic but after the developmental phase is over, treatment is much less effective in completely correcting the intellectual deficits. Impressive studies by LEVINE (1958, 1959) and by RUEGAMER et al. have shown that the stress of handling rats in infancy produces a modification in the activity of the adrenal-pituitary axis which can be detected by biochemical tests and which persists into adulthood, producing a variety of effects such as differences in weight, emotionality, and learning in certain stressful situations. HESS has evidence suggesting that meprobamate can affect the imprinting of infant ducks.

The foregoing type of observation suggests a whole new research area of utmost importance. To what extent can a transient biochemical intervention at the appropriate stage of development either intensify or retard the development of certain systems and thus have a lasting effect on subsequent behavior?

Use of test-development procedures

In addition to analytical studies, we believe that the field of psychopharmacology needs an extensive program of test-development analogous to that which has produced such important practical results in the area of aptitude testing. Many of the behavioral techniques which we have described were developed for other purposes; we need additional work to determine their applicability to the specific problem of screening drugs. As we know from experience, developing an effective battery of tests is not something that can be accomplished overnight. It is unsafe to rely on face validity, in other words, on how convincing the test looks, even to an expert! Each test must be empirically tested. Usually it is necessary to try out many more different tests than ultimately will be used. Promising tests often can be improved. For example, in the huge Air Force program in World War II more than a hundred psychologists worked for several years trying out more than a thousand both old and newly-developed tests for the selection of pilots. But the training economies resulting from this program, not to mention dividends in terms of saved lives and increased fighting efficiency, paid for many times the price of this program in the U.S. Air Force.

In developing a test one should consider a number of points:

1. Sensitivity—reliability. How reliably can the test measure differences? If the test cannot give a sensitive and reliable measure of some effect, it is not likely to be useful.

In developing some of the improved techniques described in this paper, our group has found it desirable to try out a number of variations on the basic procedure. For example, in the potentiated startle technique,

D. R. WILLIAMS found that sensitivity was greatly reduced if the trials were given too rapidly, but that spacing the trials more than 90 sec. apart did not result in enough additional improvement to justify the additional time. Furthermore, he found to his surprise that a fixed interval between trials seemed to be somewhat more effective than a variable one. It is quite possible that the technique could be improved still further by systematically testing the effects of yet other variations.

In the telescope alley we have found that a considerable number of changes apparently do not make any great difference. For example, we can run the trials at different distances in a randomized instead of a progressive sequence, or we can make the full length of the alley safe and the shorter runs progressively more dangerous. Even if we combine these two procedures, the rats still learn the basic discrimination of running fast at the safer long distance and slower at the short dangerous one without any help from the cue of the sequence in which the runs are given. But under this last set of conditions, the test seems to lose its sensitivity for a number of drugs so that their effects unaccountably disappear.

In the final example we were trying out the effects of alcohol on electrical stimulation of the brain at a point where the first effects are rewarding and the subsequent ones are aversive. The rats pressed a bar to turn this stimulation on and then rotated a little squirrel-cage wheel projecting into the apparatus to turn it off, then returned to the bar, etc. (MILLER 1957b, Fig. 8, p. 99). We chose the wheel and the bar so that the two types of responses would be quite distinctive.

The alcohol produced a considerable increase in the time the rats took to rotate the wheel to turn off the stimulation, but did not reduce the time it took them to press the bar to turn the stimulation on. Thus it seemed that the alcohol reduced the negative punishing effect on this part of the brain without greatly affecting the positive rewarding effect. This result fitted in nicely with those of our other studies. But when we reversed the function of the two devices so that rats had to rotate the wheel to turn the stimulation on and press the bar to turn it off, our results were completely reversed. Apparently alcohol interfered more with the motor skill required to stand up and rotate the wheel, than it did with that required to crawl up and press the low bar. Our results were an artifact of a side-effect of the drug. We changed the apparatus to symmetrically placed but distinctively located low black and white bars, and as an added precaution wired the apparatus so that it was easy to reverse their function by throwing a single switch.

Obviously, it is desirable to comb the bugs out of new techniques and to discover which combinations of variables will produce the best results. Although our growing theoretical understanding often can

greatly hasten the process, there still remain many possible combinations to be empirically tested. Therefore, the process is a laborious one.

But the mere fact that the test can yield a reliable measure does not mean that this will be a useful measure. At the present time some of our screening tests may be sensitive measures of only side effects that seem specific and relevant, but actually are irrelevant to the clinically useful effects of the class of drugs. Therefore, the following points must be considered also:

2. Intercorrelations. A great deal can be learned about tests by finding out how well they agree with each other under a variety of conditions. If a number of apparently different tests agree, our confidence is increased that they are measuring some reasonably stable and general factor. On the other hand, we may find that instead of dealing with a single unitary factor, we are actually dealing with a number of different factors. An example of this approach is our study comparing a number of different measures of fear.

Internal consistency among a number of tests purporting to measure the same thing is desirable. When a number of tests seem to be measuring the same thing, it is useful to select those tests which are most predictive of the general cluster of results. On the other hand, for a comprehensive battery, it is desirable to have different tests which do not correlate highly with each other, and thus measure different things instead of merely duplicating each other. In short, if tests group themselves into different clusters, it is desirable to select the best tests from each cluster. Factor analysis can help one to achieve this goal by indicating how many different factors are being measured by a battery of tests and how heavily each of these tests is weighted in the different factors. In this way a smaller number of tests can be selected which will measure the same factors that are involved in a larger battery. Ultimately, it may be desirable to make factor analyses of tests, drugs, and individual differences in the reactions of subjects.

3. Validity. The ultimate goal is to know how well the test or the battery of tests will predict some practically useful criterion. For example, we want to know how well our various screening tests will predict clinically useful effects of the drug. A program of validation can be started by using various tests on drugs already known to have clinically useful effects, along with other drugs which have similar side-effects without any clinical utility. If the battery of tests can select and differentiate among the drugs known to be clinically useful, we have more confidence in its utility with unknown drugs.

But before we can validate animal screening techniques for *new* drug effects, it may be necessary to improve the criterion measures

against which we validate the tests, or in other words, to devise more standardized, reliable and sensitive measures of the clinically useful effects on human subjects.

Finding radically new drugs

The current procedures of the drug industry are primarily adapted to finding better drugs of the types we already have. This is a worthy task and has already yielded valuable results. The techniques are not nearly as well adapted to the task of discovering radically new types of drugs. Existing tests, such as the tail-withdrawal test for analgesics, which have been found to be empirically effective but are not thoroughly understood, may be specific to a certain class of compounds such as the opiates. Similarly, the probability of finding something useful is much higher when one looks at compounds similar to those that have already been found useful. Thus, in looking for new analgesics one is likely to examine molecules which are similar to the opiates. Both this procedure, and the tail-withdrawal test, may predispose one to addictive compounds and, as WIKLER has pointed out, prevent the discovery of a non-addictive analgesic. The same general point applies to research on other categories of drugs.

With the millions of possible compounds, the probability of turning up useful drugs by an indiscriminate search is distressingly low. Furthermore, there is a possibility that some of the most psychologically useful drug effects may not appear in the lower animals with which it is practicable to put huge numbers of drugs through a coarse screen. Certain useful effects may appear only in an animal with the highly differentiated brain of man. But it is just with man that the difficulties of indiscriminate screening become greatest.

These difficulties argue strongly for the desirability of three procedures: (1) Investigation of the behavioral effects of normal biochemical processes in the body, especially the brain; (2) using the simple screens to test for any marked psychological effects of all drugs tested for any reason at the human level; and (3) taking full advantage of the enormous amount of informal testing that has been done by many millions of people in hundreds of societies for thousands of years.

We have already discussed the first of these procedures and will now deal briefly with the other two.

Improving the results of human clinical tests

Many of the drugs, such as antibiotics, which are tried out on human patients for quite other reasons, may have useful psychological effects which are not discovered by the haphazard crude observations that are now made but could be discovered by relatively small amounts of

additional systematic search. Thus, reserpine and iproniazid were used for quite a while before their psychological effects were suspected. If in their first clinical tests they had not seemed promising for other uses would the psychological effects of these drugs ever have been discovered?

We need to develop and systematically use some relatively simple but effective procedures for picking up the grosser psychological effects of any drugs that are being clinically tested for any reason. One such technique that is already available is a simple adjective check-list. The Rochester group (NOWLIS and NOWLIS) has found that such a check-list yields reliable measures of motivation and mood which agree with other more elaborate techniques such as objective measures of performance in a variety of standardized situations and ratings by skilled judges. This technique will pick up effects that are missed by cruder and more casual reports by the subjects or their associates.

We also need to improve the measures that are used in the more elaborate clinical tests of those drugs which seem promising on the basis of the first coarse screen. As far as gross intellectual or psychomotor effects are concerned, it should be relatively simple to use some already developed battery of tests, such as those that have been validated for the selection of pilots. Such a general battery would also be useful to pick up any behavioral toxicity. We should also try to develop other techniques. For example, we may find it profitable to break down the global psychiatric diagnosis into specific items of behavior which can be tallied on a check-list. A step in this direction has been made by WITTENBORN. Investigators who are familiar with aptitude testing techniques and with the problem of objectively scoring complex aspects of human behavior, such as leadership, can contribute to such a program. Finally, we need close communication between the people developing the animal tests and those securing the clinical results.

Cross-cultural canvass of folk remedies

It is well known that many useful drugs have been developed by applying modern pharmacological techniques to the investigation of old folk remedies. Though some work has been done in primitive societies, there are many cultures other than our own which remain to be systematically investigated. The fact that the scientific technology of these cultures is behind ours, does not mean that they have been any less acute over thousands of years in trying out the plant and animal products in their different environments. Because wide-scale testing for psychological effects is so desirable but difficult at the human level, there are special reasons for thoroughly canvassing for psychotropic drugs the entire cross-cultural spectrum.

The first step of surveying the known literature is made enormously easier by the existence of the Human Relations Area Files (WALKER). In these files ethnographic material from representative societies is translated into English, printed on card form, and classified so that retrieval is much more efficient than from the original material. In addition a special coordinated effort should be made to bring back more material on psychotropic drugs from anthropological field studies. Outer space and the mantle of the earth will remain, but the human laboratories provided by many "primitive" societies are rapidly disappearing for ever.

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Quantification of the EEG-Activation Response: Effects of Atropine*

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With 7 Figures in the Text

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The activation response of the EEG is characterized by a low voltage, high frequency pattern which occurs in response to a stimulation of the medial reticular formation of the brain stem either directly (electrically) or indirectly by many modes of physiological stimulation. Gibbs and Gibbs (1941) postulated a "principle of constancy" of the EEG wave forms, i. e. a tendency for slow waves to have large amplitude, and for fast waves to be of relatively low voltage. This inverse relationship between frequency and amplitude is also found, according to the same authors, in the electrical activity of smooth muscle, heart muscle and nerve. Furthermore, it has been shown by Mangold et al. (1951) that the metabolic activity of the brain is essentially constant during sleep and during wakefulness. One might therefore think of the activation response as a redistribution of the energy wave forms with a shift towards desynchronization. Whether or not there is an "increase in '*activity*'" in the sense of increase in oxygen consumption and increase in metabolic rate is a matter which cannot be settled by present day techniques. In the present study the meaning of the terms "activation response", and "increase in activity" has been taken strictly as "lack of synchronization" and "increase in temporal dispersion of the cortical potentials".

The methods employed in the analysis of clinical electroencephalograms, frequency analysis, cross-correlation techniques, histograms and so on, do not apply readily to the very restricted nature of the activation response (Khozevnikov, 1958). The clinical techniques have an inherent "broad range" coverage of the EEG spectrum which is unnecessary for the activation response phenomenon.

In the study of drug action, the primary concern is the comparison of degrees of activity and thus a quantification of the activation response and the manner in which it is modified by a given stimulus, a drug or a

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surgical procedure would provide useful information to evaluate activity level with some degree of objectivity.

Domino (1955) and King (1956) attempted to measure the threshold stimulus necessary to evoke activation response to electrical stimulation. Longo (1956) evaluated the duration of the activation response and found it to be shortened or prolonged by specific drugs. With sedative doses of depressant drugs, very subtle alterations of the EEG patterns may be produced. Under these conditions it becomes very difficult to judge how much "activation" is present or the degree of depression of the EEG. Rothballer (1956) described the intermediate stages of the activation response and pointed out the continuum of frequencies from a state of frank activation (highest frequencies, lowest voltage) to a state of complete inactivation or "synchrony" (slow frequencies, high voltage, maximum "spindle formation"). No satisfactory unit has been devised to give an objective measure of depression or enhancement of the activation response. Although the activation response phenomenon is usually obvious on visual inspection of the EEG records, its interpretation in quantitative terms has been met with several difficulties as has been discussed recently (Martin et al. 1958).

The aim of our study is to present a quantification of the activation response where two readily identifiable parameters of the EEG, frequency (F) and voltage (V) are integrated with respect to time (T) and thus permit the evaluation of "total amounts of activity" during any one of the activation responses. Control activation responses were obtained over an appropriate range of electrical stimuli and then atropine, a known depressant of the CNS and in particular of the activation response (Funderburk and Case 1951; Rinaldi and Himwich 1955; Longo 1956) was administered. The same electrical stimuli were repeated and if needed extended in intensity beyond the control range.

Methods

Twenty cats were prepared according to the *encéphale isolé* technique described by Bremer (1935). Under ether anesthesia a tracheal cannula was inserted and the femoral artery cannulated with a polyethylene catheter for the recording of blood pressure. The femoral vein was also cannulated to permit intravenous injections of the drug under study.

The anesthesia was then deepened and the spinal cord transected just posterior to the foramen magnum. At this time artifical respiration was instituted by means of a Harvard Respirator connected to the tracheal cannula and the ether was discontinued.

The animals were positioned in a Labtronics stereotaxic instrument and concentric bipolar electrodes lowered into the medial reticular

formation of the brain stem through a burr hole and a small incision of the dura mater. The electrodes were used for electrical stimulation and their tips rested in the vicinity of the red nucleus (Horsley-Clarke coordinates $A=1$; $L=2$ to 3; $V=-1$ to -2).

The electrical activity of the brain was recorded by means of phonograph needles driven into the cranial vault over the frontal, parietal and occipital regions bilaterally. Skin and muscle masses had been previously widely reflected to prevent movement artifacts.

The brain potentials were recorded with an Offner electroencephalograph simultaneously with the electrocardiogram and also with the blood pressure which was obtained with a Statham P23A strain gauge and amplified by an Offner type 131 D—C amplifier.

The electrical stimuli were delivered by a Grass stimulator: The parameters being: frequency 250 c/s; Pulse duration 1 millisecond, stimulus duration 5 seconds; the voltage was kept the independent variable.

The drug employed was dissolved in 0.9% sodium chloride solution and slowly injected via the polyethylene catheter into the femoral vein.

Analysis of data

A simple mathematical relationship between frequency and voltage of the EEG wave forms permits the quantification of the activation response in the following manner: The two parameters, frequency (F) and voltage (V), can be related in such a way so as to enhance the changes they undergo during a period of activation as compared with their values in an "inactive" or "resting" phase of record.

The product F (frequency)$\cdot 1/V$ (voltage) has been chosen and expressed in arbitrary "activation units", U_A, such that $F \cdot 1/V = U_A$.

In an ideal wave form such as is represented in Fig. 1, the process is simple: for the upper tracing the value $U_A = F \cdot 1/V$, or $10\ \text{c/s} \cdot 1/200 = 0.05\ U_A$; and for the lower tracing, U_A becomes $30\ \text{c/s} \cdot 1/50 = 0.6\ U_A$.

In an actual EEG record the value of "frequency" was obtained by counting each individual wave over a given 1-second period (equivalent to 2.54 cm length of record). Thus, in Fig. 2a, 10 waves per second were counted for the first second. The amplitude of each wave counted was measured peak-to-peak and expressed in terms of microvolts (using the 100 microvolt calibration signal as reference). The average voltage was then determined by summating the voltages of all the individual waves and dividing by the number of waves.

When a small component wave appeared on the ascending or descending phase of a larger wave it was counted if greater than 1.0 mm and rejected if less than 1.0 mm. For example, in Fig. 2b, wave No. 6

was counted while the slight deflection (arrow) just preceding wave No. 1 was rejected. When a wave was included in the frequency count its voltage was measured from preceding trough to peak. The multiplying process was performed as it has been described for the ideal sine wave form, i.e., $U_A = F \cdot 1/V$ (V being the average voltage).

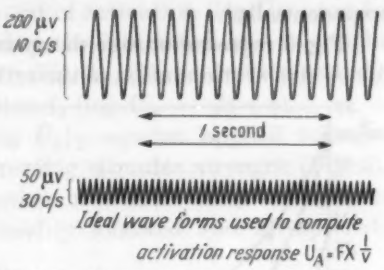

Fig. 1. The ideal wave forms represented above were obtained from an oscilloscope tracing. The upper trace: 10 cycles/second with a constant voltage (arbitrarily defined as equivalent to 200 microvolts) gives a value $U_A = F \cdot 1/V = 10 \cdot 1/200 = 0.05\ U_A$. The lower trace: 30 cycles/second with a constant voltage of 50 microvolts (one-fourth the amplitude of the upper wave) gives a value $U_A = F \cdot 1/V = 30 \cdot 1/50 = 0.6\ U_A$

The analysis was done on randomly selected 5-second intervals of record taken 2 minutes immediately preceding the activation. The number of samples analyzed varied from five to ten, depending on the variability of the predominant wave forms. Following the stimulus evoking the activation response, the record was analyzed at 5-second intervals beginning immediately at the end of the activating stimulus. The collection of samples was continued until the values of U_A had returned to prestimulus level, i.e., to the values of the control period. It might be pointed out that in any one preparation of the "*encéphale isolé*" type,

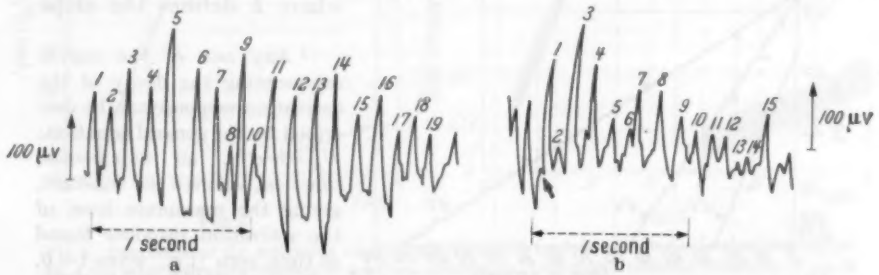

Fig. 2a and b. Illustration of the method used to compute U_A values ($F \cdot 1/V$) in an actual EEG tracing. In a, each wave of the spindle is counted as 1, or a total of 10 for the one-second period. In b, waves of more than 1 mm are counted as 1, waves of less than 1 mm are rejected. Thus, the slight deflection (arrow) preceding wave 1 was rejected, while wave 6 was included in the frequency count. Total count for the one-second period is 10. The voltage in each case is computed by summating the voltages of all the individual waves (included in the count) and dividing by the number of waves or frequency. Calibration is 100 microvolts

the EEG in the "resting state", before and after an activation response, showed a variable number of spindles per unit of time; however, the relation, $F \cdot 1/V$, remained quite constant by virtue of the averaging process. Whenever an EEG depressant drug was administered, the resting state record was shifted to lower values of U_A, but again the level remained quite stable over long periods of time. Thus, it was found that

the values $F \cdot 1/V$ (or U_A) taken during the activation response varied with respect to time, i.e. $U_A = \int (F \cdot 1/V) dt$. If the values U_A were plotted against time in seconds, a curve was constructed which decreased exponentially[1].

Fig. 3 represents three decay curves of activation responses obtained by electrical stimulation of the reticular formation. The voltages applied were 2, 4, and 6 volts. If the log values of U_A were placed on the ordinate instead of U_A, straight linear relationships were obtained (Fig. 4). Each of the curves of Fig. 4 includes an area which represents the total "amount" of activation that was evoked by a specific stimulus strength (i.e., 2, 4 or 6 volts, respectively) and each curve can be represented by an equation of the general form: log $U_A = -kt + b$ (equation 1) where k defines the slope

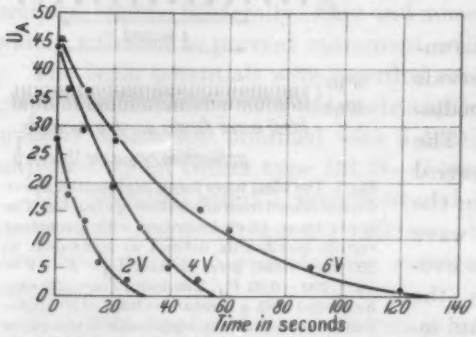

Fig. 3. Decay curves of the activation response resulting from electrical stimulation of the medial reticular formation with 2, 4, and 6 volts respectively, (square waves of one millisecond duration; 250 cycles/second). Abscissa: time in seconds. Ordinate: values of $U_A = (F \cdot 1/V)$

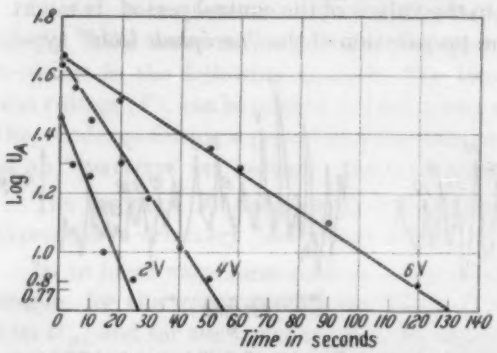

Fig. 4. Decay curves of activation response identical to Fig. 3 after log transform of U_A values. Abscissa: time in seconds. Ordinate: log of U_A values. Note that each decay curve taken with respect to the x and y axes includes a triangular area

[1] Any one of the curves representing the decay of the activation response can be described by the general equation: $U_A = b \cdot e^{-kt}$, an exponential function, where b is a constant, giving the maximum level of the activation response found at time zero (i.e., when $t = 0$, $e^{-kt} = 1$, and $U_A = b$), and k is another constant determining the slope of the decay curve.

The total amount of activation $(U_A)T$ obtained from time zero to the time t when the activity level has returned to pre-stimulus value is the integral of the equation, $U_A = b \times e^{-kt}$, or

$$(U_A)_T = \int_{t=0}^{t=t_a} (b \cdot e^{-kt})\, dt = -b \cdot \frac{e^{-kt}}{k} \Bigg]_{t=0}^{t=t_a}$$

The integration and computation of such a function is complex and tedious. The log transform of U_A, however, yields the linear equation, log $U_A = -kt + b$, which can be handled easily. The numerical values reported in the present study were obtained with the latter equation.

of the curve and b is a constant such that at $t=0$, log U_A is maximum and equals b. The total amount of activation then is equal to the integral of equation 1.

The computation of the total amount of activation (log $U_A)_T$ can be performed quite easily by observing that the integrated area is a right triangle of known height (h) and base (b) (Fig. 4). The area of the triangle then is equal to the integral of equation 1, (log $U_A = -kt + b$).

Plotting the integrated values (log $U_A)_T$ against applied voltages gives a curve rising linearly with increasing stimulus strength (Fig. 5).

Administration of drugs or alteration in stimuli bring changes in activation responses which can be readily followed and interpreted.

Results

As an example of the application of the method, the activation response to electrical stimulation of the medial reticular formation was computed in an untreated animal and in the same animal after the intravenous injection

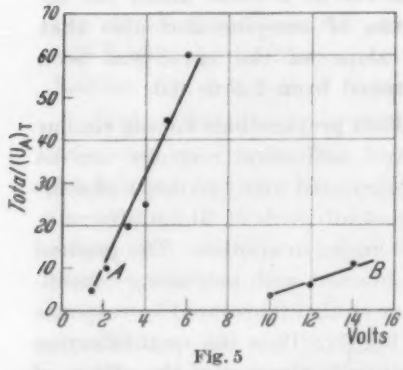

Fig. 5

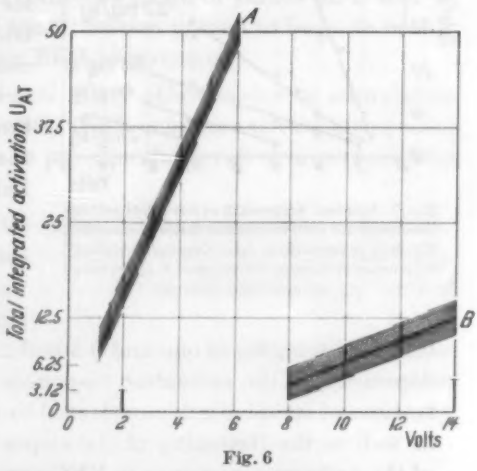

Fig. 6

Fig. 5. Depression of the activation response obtained with intravenous administration of atropine. In A the total activation response to electrical stimulation of the medial reticular formation, U_{AT}, is plotted against each corresponding voltage of the applied stimulus. In B the same animal after receiving 2 mg/kg of atropine. There is a marked elevation in threshold for the activation response, from 1.5 to 10 volts and a flattening of the slope of the curve (from 2.3 to 0.4)

Fig. 6. The depression of the activation response by atropine administered intravenously. Curve A represents the average activation response to electrical stimulation of the medial reticular formation in 4 cats. In B, the same animals after administration of 2 mg/kg of atropine. The threshold of the activation response has been elevated from 1 to 8 volts, and the slope of curves decreased (from 2.5 [A] to 0.5 [B]). The average standard deviations, 2.95 U_{AT} for A and 4.1 U_{AT} for B, appear as vertical lines on the abscissa

of 2 mg/kg of atropine sulfate. The results are plotted in Fig. 5. The depressant effects of atropine on the activation response in the EEG is apparent from the increase in voltage from 1.5 to 10 volts needed to obtain a post-stimulus activation response at all, and in the flattening of the slope of the curve (from 2.3 to 0.4).

The mean values for such a depression were established in 4 animals (Fig. 6). The control activation response to electrical stimulation of the reticular substance of the brain stem is represented by curve *A* where the average standard deviation between animals is 2.98 U_{AT} for a mean reading value of 28.3 U_{AT}, denoting the reproducibility of this method of analysis. Curve *B* depicts the activation response of the same animals after they had received 2 mg/kg of atropine intravenously. The average standard deviation between the activation responses after atropinization is 4.1 U_{AT} for a mean reading value of 6.7 U_{AT}, indicating larger variations from animal to animal within the group, as compared to the untreated one.

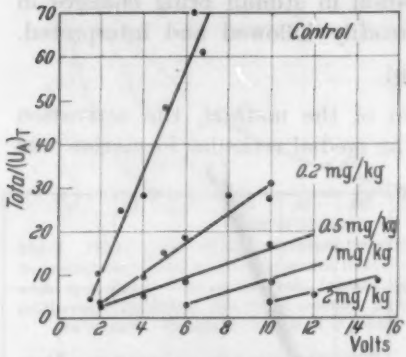

Fig. 7. Gradual depression of the EEG activation response with increasing doses of atropine. The four preparations had identical "control" activation responses throughout a given range of electrical stimuli

The comparison of the two curves demonstrates that the threshold for the activation response to electrical stimulation of the medial reticular formation has been shifted from 1.5 to 8 volts under the influence of atropine and also that the slope of the curve has been flattened from 2.5 to 0.5.

Four preparations having similar control activation response curves were injected with two doses of atropine at intervals of 30 minutes; e.g. 0.2 and 1.0 mg/kg in one and 0.5 and 2.0 mg/kg in another. The gradual depression of the activation responses obtained with increasing concentrations of atropine is demonstrated by the shifts in threshold for response as well as the flattening of the slopes (Fig. 7). Thus the quantification of the activation response in EEG permits a distinction of the effects of different concentrations of a known CNS depressant drug and also allows the construction of dose-response curves.

Discussion

The results obtained with atropine in the present study indicate that the effect of such a drug on the CNS can be estimated objectively by the quantification of the activation response.

The calculation is based on the equation $U_A = f\ (F \cdot 1/V)$ which permits the computation of instantaneous EEG "activity levels" in terms of U_A (units of activity).

The two parameters, F and $1/V$, give the individual values of U_A within certain limits: F is relatively unrestricted and follows the theorem of direct proportion, the higher the frequency (F), the higher will be the corresponding U_A for any given constant value of $1/V$. As for the voltage

(V), when the EEG tracing tends towards an isoelectric line, then the parameter $1/V \rightarrow 1/0 \rightarrow \infty$. Thus, the entire function $F \cdot 1/V$ would reach "infinite" values at a time when there is no electrical activity, although F has become nearly 0 simultaneously.

The relation, $U_A = f(F \cdot 1/V)$, has been applied to the EEG records of all the "*encéphale isolé*" preparations of this study. Very reproducible results were obtained because of the relatively narrow fluctuations of the numerical values of F (frequency) and V (voltage). The range of variations were respectively, F: from 2 to 35 c/s and V: from 50 to 300 microvolts (and on very rare occasions, 500 microvolts). If, however, both F and V were to reach very large values, such as would be found in a grand mal seizure record, the activation units U_A would no longer be comparable to the neuronal activity existing prior to the seizure. The constancy of energy output postulated by GIBBS obviously does not hold in pathological states. While within the restricted range of values for F and V, described above, this method of quantification proved to be quite useful, it may be of little value for other EEG phenomena.

It was found that electrical and many other modes of stimulation can elevate the values of U_A abruptly (such as in the activation response) which then slowly decay (return to pre-stimulus level) in an exponential fashion as a function of time.

It must be pointed out that the activation responses have to be maintained within "finite" limits of time in order to obtain useful and meaningful information from the analysis. It is obvious that if the upper limit of the integral, $t = ta \rightarrow \infty$, the values $(\log U_A)_T$ also $\rightarrow \infty$. Thus, it was found that activation responses lasting less than 10 and preferably less than 5 minutes, were optimal to be processed by the quantification method. This conditional temporal restriction can always be met by careful selection and application of the activating stimulus.

The quantification of the activation response was always begun immediately after the end of the stimulation period. It was observed that during the period of stimulation the EEG record may show high frequency signals superimposed on the accelerated pattern of activation, and that these artefacts disappear after the stimulus is turned off. Inasmuch as the period of stimulation was kept constant and of short duration, 5 seconds, it was decided to measure the U_A values for the activation response after the end of the stimulation period, when no artefacts were possible.

The selection of a portion of an EEG record representative of "average activity" at any one time is of course subject to error; it was found that the selection of a larger number of samples always permitted calculation of reproducible estimates of the changes taking place in frequency and voltage amplitude in any part of the record.

The occasional spurious shift in base-line, which is usually due to faulty electrical equipment or other source of artefact, such as movements of the animal can be overcome by a simple re-alignment of the record or by choosing, if available, another portion of the tracing where there is no shift.

It must be emphasized that this method of quantification of the activation response in the EEG is only a relative measure of the activation response and that the subject acts as its own control. There are no absolute values of U_A. In all cases the activation response was estimated in terms of rise in U_A values from the control period, measured immediately preceding the application of the stimulus, and in terms of the duration of the activation response proper. The return to U_A values equal to those of the pre-stimulus period were the index of the end of the activation response.

Comparisons between animals may show variations which are inherent in their different physiological states.

As far as the activation response *per se* is concerned, it can be evoked by many different modes of stimulation (electrical, sensory, pharmacological) and still yield useful results providing that the "stimulating" agents can be graded in intensity and adequately measured. In that respect the ease with which electrical stimulation of the medial reticular formation can be applied in discrete measurable quantities made it the stimulus of choice. There is a direct correlation between the strength of the electrical stimulus and the duration of the response and although electrical stimulation has some disadvantages, the high frequency artefacts for instance, it remains the *modus operandi* with which highly reproducible results were obtained for single preparations and even among different animals.

The quantification of the activation response in the EEG has proved to be a useful way of assessing the effects of drugs on the electrical pattern of the cat's brain. Different drugs and different concentrations of a single drug have been differentiated in their depressant effects on the activation response to electrical stimulation of the midbrain reticular formation.

The method has been applied in the study of other CNS depressants, methatropine, scopolamine, pentobarbital and chloropromazine as well as of drugs capable of evoking the activation response (PAUL, et al. 1959).

Summary

The activation response of the EEG can be modified by various drugs, but the quantitative evaluation of the changes is difficult with conventional methods of EEG analysis.

A simple mathematical relationship between frequency (F) and voltage (V) of the EEG record permits the definition of a unit of activation U_A such that $U_A = f\,(F \cdot 1/V)$ which in turn varies as a function of time (t).

Integration of U_A with respect to time yields "total amounts" of activation for a given activation response.

It has been shown that there is a linear relationship between the strength of an applied electrical stimulus to the medial reticular formation of *encéphale isolé* cat preparations and the integrated activation response: to increasing voltages correspond increasing activation responses.

Atropine, a known CNS depressant, was administered intravenously to the same preparations.

Plotting the various voltages against the integrated activation responses before and after atropine, yielded dose-response curves thus quantifying the depressant effect of atropine in different doses on the EEG activation response.

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Effects of Aggregation and Temperature on Amphetamine Toxicity in Mice*

By

RUDOLF HÖHN** and LOUIS LASAGNA

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Introduction

GUNN and GURD observed that grouped mice were more susceptible to the excitatory effects of amphetamine and allied drugs than single mice. CHANCE (1946) estimated the LD 50 of adrenaline, methedrine, ephedrine and amphetamine under varying degrees of confinement and aggregation. When the confining area was kept constant and the number of mice increased from one to 10, the toxicity of adrenaline was doubled and that of amphetamine was increased nearly tenfold. The increase of mortality by ephedrine and methedrine fell between these extremes. Most of these studies were carried out at a temperature of 80° F. A decrease of temperature to 60° F markedly reduced the effects of aggregation on the toxicity of amphetamine, the lethality of the drug dropping almost to the figure obtained with solitary mice under the same conditions. The animals were almost inactive at 60° F.

CHANCE (1947) also investigated other factors influencing the toxicity of sympathomimetic amines, including amphetamine, in mice. Variables increasing the toxicity were: higher weight of the mouse, environmental noises, confinement of the area in which the animal was placed, and aggregation (the presence of other mice). The latter was felt to be the factor having the greatest effect under conditions normally employed in pharmacological laboratories. Increased hydration and particularly lowering of environmental temperature proved to be protective. Although the strain of mouse was found to be important, sex seemed unimportant. The intensity of illumination and the transparency of boundary walls did not alter the results.

From his observations CHANCE concluded "that the essential feature of these amines is that, unlike other central nervous stimulants, they induce in the mouse a state of excitability rather than of excitement.

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Lethal doses almost invariably kill the solitary mouse as a result of violent convulsions, which, therefore, may appear when the excitation in the central nervous system reached a critical level. Doses themselves not lethal may thus become so as a result of external factors raising the excitation to a critical level."

LASAGNA and MCCANN confirmed CHANCE's observations that amphetamine is more toxic in aggregated mice. They postulated that the agitated mouse in a crowded environment, suffering deleterious effects from the proximity of agitated mouse neighbors, might benefit from "tranquilizing" drugs. To the single mouse pentobarbital, phenobarbital, and chlorpromazine conferred no or only slight protective effect against the toxicity of amphetamine. In grouped mice, pentobarbital had no effect on the LD 50. A marked protection, however, was achieved with phenobarbital at doses producing ataxia and „sleepiness" while with chlorpromazine a significant protective effect was observed in doses well below those producing unconsciousness. Reserpine and promazine were also found protective, the latter, however, less so than chlorpromazine.

It appeared of interest to investigate in greater detail the interaction of confinement, aggregation, and temperature on the toxicity of amphetamine in mice. An attempt was made to discover any quantitative relations existing between those variables when other factors were kept as constant as possible.

Methods

Wire mesh cages of various sizes were used to vary the confining area. Male Swiss albino mice of 18 to 24 g weight obtained from Research Supply Co., Philadelphia, were used. Before experiments animals were kept in an animal room lit by daylight during the day and kept dark at night. To avoid possible variations due to the physiological daily rhythms in mice described by HALBERG et al., the experiments were usually started at 10 a.m. and stopped at 4 p.m. Single mice and mice in groups[1] of 3 were placed in wire mesh cages with floor areas of 25, 56, 100, and 185 cm^2; Animals in groups of 5 were placed in cages with floor areas of 100, 185, and 500 cm^2. The wire mesh allowed for adequate observation of the animals and dissipation of any local increase in temperature within the cage due to body heat. The mice were injected intraperitoneally with racemic amphetamine sulfate (Benzedrine sulfate solution, 20 mg/ml, SKF) in a volume of 0.25 ml per 20 g mouse. The amphetamine was diluted in 0.9 per cent NaCl solution. Mice were placed in an air-conditioned room (23° C to 25° C, also referred to as "room temperature"), or in a cold room (8° C to 12° C). The usual temperatures for these rooms were 25° C and

[1] The mice were grouped so as to combine mice housed prior to the experiment in different cages (10 to a cage).

10° C respectively, and both were artificially lighted. In most instances mice were injected at room temperature with a given dose and then randomly allocated either to the 25° C or to the 10° C environment. Some of the animals, however, were injected immediately after being placed in the cold. The mice were observed for 5 hours after injection and the number of dead animals recorded at hourly intervals. The dosages of amphetamine given to single animals were 10, 25, 50, 100, and 150 mg/kg, whereas the grouped mice received those doses and also 200 mg/kg. For evaluation of the effects on amphetamine toxicity of shorter exposure to cold, some mice were injected at 10° C and then moved to 25° C after varying periods (from 5 to 180 minutes) of exposure to cold. For comparison, groups of mice, injected with same dosage as these animals, were simultaneously observed at 25° C.

To estimate the effects of "pre-treatment" with cold, mice were exposed to 10° C for 30 minutes, then moved into room temperature and injected immediately with amphetamine. The results were compared with mice simultaneously injected but not "pre-treated" in the cold.

In one experiment on 24 mice injected with 150 mg/kg and placed 3 per container, the time from injection to death was measured with a stop watch over the first 30 minutes.

Uninjected single and grouped mice were exposed for 5 to 6 hours to 10° C to evaluate the effects of cold on non-medicated animals.

Results

Aggregation (see Table 1). At amphetamine doses of 25 to 100 mg/kg, at 25° C, fewer mice died when kept individually than when mice were grouped in threes or fives. At 10° C, however, no such deleterious effect of aggregation was demonstrated. Increasing the number of mice per group to five did not accentuate the aggregation effect beyond that seen with three animals per group.

Confinement. The results of decreasing the space for individual mice from 185 to 25 cm^2 had little influence on amphetamine mortality, except possibly for the cold experiment at the 5th hour, where the smallest containers showed the highest mortality (see Table 2). In the case of the grouped mice, the results of decreasing the space available per mouse yielded inconsistent data (see Tables 3 and 4), although such trends as were seen were for the most part in the direction of an inverse relationship between mortality and container size.

Temperature. A baseline study on the effects of prolonged 10° C ambient temperature indicated that unmedicated mice kept singly ($N = 24$), 3 per cage ($N = 48$), or 5 per cage ($N = 40$) were little affected by this degree of cold. Of these 112 animals, only 2 died, both in the 5th hour. The other mice showed no obvious ill effects.

Table 1. *Effect of temperature on amphetamine toxicity in single and grouped mice* (% cumulative mortality)

	3rd hour, 25° C			3rd hour, 10° C		
	1/c*	3/c**	5/c***	1/c	3/c	5/c
10 mg/kg	0	4.2	0	0	4.2	0
25 mg/kg	12.5	50.0	43.3	0	4.2	0
50 mg/kg	8.3	43.8	60.0	4.2	6.2	10.0
100 mg/kg	12.5	29.2	33.3	8.3	4.2	4.4
150 mg/kg	70.8	72.9	85.0	37.5	29.2	48.3
200 mg/kg	—	95.8	96.7	—	87.5	86.7
	4th hour, 25° C			4th hour, 10° C		
	1/c	3/c	5/c	1/c	3/c	5/c
10 mg/kg	0	4.2	0	8.3	4.2	0
25 mg/kg	12.5	50.0	43.3	12.5	8.3	3.3
50 mg/kg	25.0	62.5	66.7	12.5	22.9	30.0
100 mg/kg	25.0	54.2	40.0	33.3	41,7	13.3
150 mg/kg	79.2	75.0	88.3	62.5	41,7	51.7
200 mg/kg	—	95.8	96.7	—	91.7	93.3
	5th hour, 25° C			5th hour, 10° C		
	1/c	3/c	5/c	1/c	3/c	5/c
10 mg/kg	0	4.2	0	16.7	8.3	10.0
25 mg/kg	12.5	50.0	43.3	33.3	29.2	10.0
50 mg/kg	29.2	62.5	73.3	50.0	50.0	63.3
100 mg/kg	29.2	62.5	50.0	62.5	58.3	40.0
150 mg/kg	79.2	77.1	88.3	79.2	45.8	55.0
200 mg/kg	—	95.8	96.7	—	91.7	93.3

* 1/c: Single mice. For each dose level, 24 mice were employed.

** 3/c: 3 mice per cage. For each dose level, 24 mice were employed except 50 and 150 mg/kg, where 48 were used.

*** 5/c: 5 mice per cage. For each dose level 30 mice were employed except 150 mg/kg where 60 were used.

Table 2. *Influence of the size of the cage on the toxicity of amphetamine* (% cumulative mortality)

Single mice

	I*	II**	III+	IV++
Temperature 25° C				
Hour 4	20.0	36.7	26.7	30.0
Hour 5	23.3	36.7	30.0	30.0
Temperature 10° C				
Hour 4	33.3	23.3	20.0	26.7
Hour 5	66.7	53.3	40.0	33.3

Pooled data for all doses (amphetamine 10—150 mg/kg i.p.) (n = 30 per group).

* I: confining floor area of 25 cm².

** II: confining floor area of 56 cm².

\+ III: confining floor area of 100 cm².

\++ IV: confining floor area of 185 cm².

Table 3. *Influence of the size of the cage on the toxicity of amphetamine* (% cumulative mortality)

3 mice per cage

	I*	II**	III+	IV++
Temperature 25° C				
Hour 4	68.7	58.3	64.6	47.9
Hour 5	70.8	62.5	66.7	47.9
Temperature 10° C				
Hour 4	45.8	33.3	31.3	27.1
Hour 5	60.4	39.6	43.7	45.8

Pooled data for all doses (amphetamine 10—200 mg/kg i.p.) (n = 48 per cage size).

* I: confining floor area of 25 cm².

** II: confining floor area of 56 cm².

\+ III: confining floor area of 100 cm².

\++ IV: confining floor area of 185 cm².

For *single* amphetamine-treated animals, it appeared to make little difference whether the ambient temperature was 25° C or 10° C, for the first three hours. Over this period of time, the only impressive difference was at the 150 mg/kg dose, where the mice kept at room temperature showed a higher mortality than those at 10° C. By five hours, however, most doses studied produced a higher mortality in the cold (see Table 1).

For *grouped* animals, on the other hand, a temperature of 10° C provided substantial protection against most dose levels of drug over the first 3 hours, but by the end of five hours, the difference in mortality between the mice kept in the cold and those kept at room temperature had narrowed appreciably (see Table 1).

Table 4. *Influence of the size of the cage on the toxicity of amphetamine* (% cumulative mortality)

5 mice per cage

	III*	IV**	V***
Temperature 25° C			
Hour 4	67.1	62.8	51.4
Hour 5	68.6	65.7	54.3
Temperature 10° C			
Hour 4	33.3	32.0	34.7
Hour 5	52.0	37.3	49.3

Pooled data for all doses (amphetamine 10—200 mg/kg i.p.) (n = 70 per cage size at 25° C and 75 per cage size at 10° C).

* III: confining floor area of 100 cm². ** IV: confining floor area of 185 cm². *** V: confining floor area of 500 cm².

Ambient temperatures colder than 10° C (see Tables 5 and 6) increased the toxicity of amphetamine for grouped animals over that observed at 25° C.

It would appear that the grouped animals do not need to stay in the cold to obtain protection against amphetamine, since even a 10 to 15 minute exposure to 10° C *after* drug injection (see Table 7) or a 30 minute exposure to 10° C *before* drug injection (see Table 8) produced mortality rates lower than those seen in mice kept at 25° C throughout.

Behavior of mice. *Non-medicated grouped mice.* a) At 25° C mice placed into the cages appeared excited at first, ran around and made attempts to escape from the cage. Occasionally some animals fought but usually they quieted down quickly, sat, cleaned themsleves or ran around sporadically. Their activity was not as abrupt or spontaneously violent and "purposeless" as that seen in medicated animals.

b) In the cold the mice quieted down quickly, manifested tremor and piloerection. They crowded together and seemed thus perhaps to protect themselves mutually from the cold. After 5 to 6 hours a few animals manifested ataxia and marked slowing down of movements, but they recovered within 20 to 30 minutes after being returned to room temperature.

Medicated grouped mice. a) At 25° C mice became extremely restless about 5 minutes after injection; they ran all over the cage, squealed, ran into each other and frequently took the "apprehensive position", although fights were not too frequent and lasted for only brief periods. The ani-

mals showed gross tremor. Movements were very abrupt and sudden violent outbursts of motor activity occurred frequently. Salivation occurred, and the respirations deepened. Some animals had a general flaccidity of musculature and paresis of the hind legs. Occasionally mice would chew their thoracic skin until bleeding occurred. Chewing of the ears and skin of the head of other animals was seen in only a few instances. With increase of dosage, behavior became more disorganized. Up to 50 mg/kg some cooperation between animals was observable, such as for instance mutual cleaning. At high doses the animals ran into each other as if they could not see, and jumped against the walls of the cage. After 5 hours, the surviving animals had a tremor, appeared exhausted and had a marked increase in sensitivity to external stimuli such as noise, although, if not disturbed, they were not particularly overactive. When a larger group of such animals was put together in one containe, they became overtly excited again.

Table 5. *Effect of 7° C temperature on mice injected with amphetamine* (% cumulative mortality)

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Temperature 25° C for 5 hours					
50 mg/kg	0	0	12.5	16.7	16.7
100 mg/kg	0	12.5	16.7	20.8	29.2
Temperature 7° C for 5 hours					
50 mg/kg	0	0	0	33.3	66.7
100 mg/kg	4.2	8.3	33.3	83.3	100.0

Comparison between the mortality of aggregated mice (3 mice per cage) injected with amphetamine 50 mg/kg and 100 mg/kg i.p. and exposed to 25° C and 7° C (n = 24 per group).

Table 6. *Effect of 3° C temperature on mice injected with amphetamine* (% cumulative mortality)

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
25° C	0	0	29.2	52.4	62.5
3° C	50.0	91.7	95.8	95.8	95.8

Amphetamine 100 mg/kg i.p., 3 mice per cage. Animals remained at given temperatures for 5 hours (n = 24 per group).

b) In the cold, aggregated mice were much quieter than at room temperature and, except at the highest doses, activity ceased almost completely. The lower the dose, the more pronounced was the tendency to crowd together. Marked tremor and piloerection were present. At high doses hyperactivity prevented any effective huddling. Chewing of the wire of the cage walls, which was also observable at room temperature, was so strong in the cold, that some mice, who pushed their snouts through the wire mesh, developed edema of the snout and could not retract it. Rather frequently stronger animals chewed the ears and skin of the head of weaker animals.

Behavoir of single animals. Single animals remained much quieter than the grouped ones. The difference in behavior was more a quantita-

Table 7. *Effect of amphetamine on mice kept at 10° C for less than 5 hours*
(% cumulative mortality)

		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Expt. I	5 hr. 25° C (n = 48)	70.8	70.8	72.9	75.0	77.0
	5 hr., 10° C (n = 48)	27.0	27.0	29.1	41.6	45.8
Expt. II	5 hr. 25° C (n = 24)	83.3	83.3	87.5	87.5	87.5
	45 min, 10° C (n = 24)	54.1	54.1	54.1	54.1	66.6
Expt. III	5 hr. 25° C (n = 72)	75.0	75.0	77.7	81.9	86.1
	10—15 min, 10° C (n = 72)	30.5	31.9	36.1	44.4	45.8
Expt. IV	5 hr. 25° C (n = 96)	44.7	45.8	51.0	52.0	54.1
	5 min, 10° C (n = 96)	42.7	42.7	43.7	44.7	47.9

For every experiment 3 mice per cage were used and amphetamine 150 mg/kg i.p. given. Mice injected at 10° were kept at that temperature for the length of time indicated, and then moved to 25° C. Mice at 25° C remained at this temperature for 5 hours.

Table 8. *Effect of "pre-treatment" with cold on the toxicity of amphetamine*
(% cumulative mortality)

	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5
Amphetamine 100 mg/kg i.p.					
Not "pre-treated"	23.3	36.7	53.3	66.7	66.7
"Pre-treated"	0	13.3	46.7	66.7	66.7
Amphetamine 150 mg/kg i.p.					
Not "pre-treated"	63.3	70.0	73.3	76.7	76.7
'Pre-treated"	40.0	40.0	40.0	50.0	56.7

In all instances 5 mice per cage were employed. "Pre-treatment" with cold had a duration of 30 minutes at 10° C; then the mice were moved to 25° C and injected immediately (N = 30 per group).

tive than a qualitative one except for those activities possible only in the social situation. Great excitement was easily provoked by external stimulation.

Death of mice. The pattern of death was distinctly different in cold and room temperature and for large and small doses of amphetamine. In room temperature, at 150 mg/kg and 200 mg/kg, most mice that died did so within the first 20 minutes with a median survival time of 8 minutes. A few minutes after the injection the animals became almost immobile, then took some ataxic steps and suddenly began to jump against

the cage walls. In most instances tonic and clonic convulsions followed and they could be provoked by loud noises or by touching the animal. A few mice died without having had convulsions and they manifested slow gasping breathing before death. Up to 100 mg/kg most animals died between the second and fourth hours. At these lower doses, the animals went through a period of "exhaustion", sometimes showing a marked weakness of the hind legs, and finally demonstrating gasping breathing shortly before death. Most animals "faded away", and convulsions were rare.

In the cold, some of the animals on very high doses (150 to 200 mg/kg) died within the first hour in convulsions similar to those of mice at room temperature. The remaining mice, and animals at lower doses, died much later. The movements became slow, their reactions to external stimuli were markedly decreased and finally nearly absent, they displayed ataxia, lost the righting reflex and showed muscular weakness. Eventually they lay immobile with gasping breathing of low frequency. They remained in this condition sometimes for 1 or 2 hours until they died. Only by close examination was one able to see whether such an animal was dead. When such an animal, appearing almost dead, was moved into room temperature it began to walk with an ataxic gait after a short time; such mice had a tendency to crowd together in a corner and remain there quietly. Gradually they became more active and finally hyperactive. Some of those animals died later on the same day, but it was impossible to predict the outcome in an individual animal. Other mice, which were practically immobile in the cold, but not moribund, became excited and hyperactive when placed at 25° C and exhibited a pattern of behavior similar to that of animals which had been at room temperature throughout.

Discussion

CHANCE's data (1946, 1947) have been in large part confirmed by our studies, but certain differences emerge, andthe whole problem of the toxicity of amphetamine in regard to aggregation, confinement, and temperature seems more complex than suggested by CHANCE. In the study of LASAGNA and MCCANN, it would appear that the toxicity of amphetamine for single animals was similar to that observed for single animals in the present study, despite the fact that the average temperature in the earlier study was somewhat higher than the temperature employed as "room temperature" here. This latter fact (taken in conjunction with the inability of body heat to dissipate in the metal cans used in the earlier study) probably explains the somewhat higher mortality seen in the grouped animals by LASAGNA and MCCANN.

Certainly the presence of more than one mouse per container increases the toxicity of amphetamine at room temperature whereas it

does not so increase mortality at certain colder temperatures. Furthermore, it would appear that the use of any "LD 50" values to express the drug effects for grouped animals versus single animals is misleading, since the dose-response curves are more regular and different in shape for single animals than for grouped animals. This latter fact is not surprising, since the failure of grouped animals to die simultaneously from a given dose of drug means that for early periods of observation one actually has several animals interacting in a container, whereas at later times there may be only one live animal remaining.

The question of confinement, i.e., restriction of space per animal, is also far from simple, except for experiments where all of the space in a container is available for one animal. With two or more animals, it is almost impossible to define how much space each animal has. Obviously, with a large "container" (an experimental room, for example) ten mice would really have essentially unlimited space and one could hardly talk of each mouse having "one-tenth of a room." There probably must be a certain minimal confinement for any given number of animals before one can talk sensibly of any degree of „crowding." Attempts to analyze for area effects by dividing the number of animals into total area available become extremely difficult, and the problems are compounded when animals in a group begin to die off. Our data lend little support to the notion that extreme confinement of *single* animals increases amphetamine toxicity, and only slightly more to the importance of confinement for grouped animals.

Ambient temperature, too, presents complex problems. Within certain limits, cold appears to have a protective effect against amphetamine toxicity, most marked in the grouped animals. But if the temperature is low enough, or exposure to cold maintained long enough, the "stress" of cold appears to augment the toxicity of the drug.

CHANCE (1947) assumed that all mice dying of the effects of amphetamine did so in convulsions and that external stimuli increased the excitability and thus lowered the convulsive threshold of the animals. In the literature two different kinds of death are described in various animal species. EHRICH et al. (1937, 1939) reported that rats "faded away" without convulsions and that some rabbits and monkeys "faded away" while others died in convulsions. SCHULZ and DECKNER gave metamphetamine to white mice and observed death in exhaustion due to respiratory arrest. In our study, mice injected with amphetamine up to 100 mg/kg died predominantly a slower death with signs of exhaustion, and convulsions were rare. Doses of 150 mg/kg produced in most animals death within the first 20 minutes and predominantly in violent convulsions. This suggests that high doses of amphetamine provoke convulsions, but that the mode of death at lower dosages and in mice which do not have convulsions is due to other toxic effects of the drug, effects

which appear considerably increased by the excitatory effects of aggregation. An interesting toxic reaction is the paresis of the hind legs in many animals, particularly at high dosages, a phenomenon described in rabbits by EHRICH et al. Self-mutilation, such as chewing of the thoracic skin, has been reported previously after amphetamine in rats (EHRICH and KRUMBHAAR) and rabbits (EHRICH et al.) and after metamphetamine in mice (SCHULZ and DECKNER).

There is a considerable variation in mortality from amphetamine, particularly in grouped animals, even when mice appear to be of similar weight and age and ambient temperature and other conditions are kept reasonably constant. This fact is well demonstrated in the results on protection of animals by exposure to cold for varying periods of time. It will be seen in the experiment in which animals were kept in the cold for five minutes and then transferred to room temperature, that the control animals, i.e., those kept et room temperature for the entire period, showed a mortality after amphetamine that was considerably lower than the mortality in other control groups in the same series of experiments (see Table 7). We consider the relationship between the mortality of simultaneously compared groups more meaningful than the absolute mortality of the mice in any given experiment. Reasons for the variability in grouped animals have been discussed above.

Cold would appear to have a sedating and quieting effect on animals, as evident both in the behavior of unmedicated animals put in the cold and in the behavior of medicated animals transferred from cold to room temperature. Death from amphetamine at high doses at room temperature appears to result from a different physiological train of events than does death from lower doses at room temperature. In addition, death from amphetamine in the cold is by and large a slower phenomenon and probably a different one from either of the types of death seen at room temperature. The protective influence of cold against the early death with convulsions in single animals does not allow any definite conclusions to be drawn because of the multiple changes which occur simultaneously in an organism under such conditions. The complexity of the problem has been reviewed by FUHRMAN.

Summary

1. White mice were injected with amphetamine sulfate in doses ranging between 10 and 200 mg/kg, and placed into wire mesh cages of various sizes. Identical groups were kept in a temperature of 25° C and in a cold room of 10° C for a period of 5 hours. The number of dead animals was registered at hourly intervals.

2. At room temperature aggregation increased the toxic effects of amphetamine. Confinement had less influence on mortality. In-

creasing the number of animals from three to five per container did not cause a significant difference in the results.

3. The influence of cold on the toxicity of amphetamine was complex. In the first few hours, a temperature of 10° C had a definite protective influence in grouped animals and a slight one in single animals. Later, this protection was lost in varying degrees. Temperatures lower than 10° C increased the toxicity of amphetamine. A short exposure of the mice to 10° C, either before or after the injection of amphetamine, had a protective influence.

4. The behavior of the animals is described. Cold had a "sedating" and "quieting" effect on non-medicated mice and counteracted the excitation caused by amphetamine. Death from lower dosages of amphetamine was distinctly different from that at high dosages, the latter type of death being preceded by convulsions. In the cold, death was preceded by a long period of moribundity.

5. Death from amphetamine is a complicated phenomenon, and the toxic effects of the drug affected by many variables. Interactions become more complex in studies of grouped animals, where greater variability in response can be expected. It would appear important for investigators reporting results with drugs of this class to describe in sufficient detail the conditions obtaining in their experiments.

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Impairment by Lysergic Acid Diethylamide of Accuracy in Performance of a Delayed Alternation Test in Monkeys*

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With 4 Figures in the Text

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Introduction

One of the properties of lysergic acid diethylamide (LSD) is the remarkable ability of extremely small doses of this semisynthetic (about 0.001 mg/kg) to produce psychological changes in human beings, including hallucinations and delusions, as well as profound impairment of performance in intellectual tests (JARVIK, ABRAMSON, and HIRSCH, 1955). Other mammals and birds require many times the human dose in order to exhibit behavioral changes (BLOUGH, 1957; COOK and WEIDLEY, 1959). In monkeys, EVARTS (1958) has shown that visual discrimination performance was not affected by doses of LSD as large as 9.95 mg/kg, but that delayed response performance was depressed. He noted that the LSD effect disappeared with continued testing, and he attributed this to overtraining. On the other hand, FUSTER (1957) has shown that performance on a tachistoscopic task by monkeys could be disturbed by doses of LSD as small as 0.005 mg/kg.

Performance on both delayed response and delayed alternation tasks has been shown to be impaired by frontal lobe and basal ganglia lesions in monkeys, but relatively unaffected by temporal lobe lesions (CHOW, HUTT, 1953; ROSVOLD, MISHKIN, and SZWARCBART, 1958). Only a few experiments have dealt directly with the effect of drugs upon the performance of intact animals in delayed response tasks (HALL, WARREN, and HARLOW, 1955) although a number of investigations have dealt with the effects of barbiturates and amphetamine upon the deficit in delayed response produced by frontal lesions (WADE, 1947; BLUM, CHOW, and BLUM, 1951; and MISHKIN and PRIBRAM, 1953).

The present experiment was undertaken to determine whether LSD would impair delayed alternation performance in the monkey, to establish the presence or absence of tolerance, and to compare LSD with several other drugs in the same test situation. It was of particular interest to determine whether the confusional properties of LSD were related to a depressant action of this drug upon behavior generally, as reflected in

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rate of lever pressing, or whether the dichotomous choice situation of the delayed alternation task might be more sensitive than overall pressing rates.

Subjects. Eight immature Macaque monkeys were used in this experiment. They had some prior experience in the test situation and had been employed in a drug experiment several months previous to the present study. Their weights ranged from 3.4 to 5.8 kilograms.

Apparatus. The principal apparatus consisted of a modified refrigerator cabinet in which the monkeys performed the delayed alternation test. On one inner wall, were two plexiglass panels, each 10 cm $\times$ 10 cm and separated from one another by a distance of 3 cm. Timing and sequence control was obtained with appropriate relay circuitry and performance was recorded on counters which registered correct and incorrect responses. While being tested, the animals were restrained by a plastic collar in a chair constructed of aluminum and masonite as illustrated in Fig. 1. As can be seen, the animal when placed in the chamber was confronted by two panels and the reward dispenser, a flexible metal tube. The chamber was ventilated by a small blower which acted also as a source of masking noise. The animal was placed in the chair just before being tested and was returned to his home cage immediately afterwards.

Procedure. All animals were pretrained daily for several weeks on the delayed alternation task prior to drug administration. After being deprived of water for approximately 20 hours, the monkey was placed in the testing chamber. The correct response on the appropriate panel was rewarded by 0.03 cc sweetened water. At the same time, this response caused an overhead light (the "go" signal) to be extinguished and started the delay period. At the end of the delay interval (2 seconds), unless otherwise stated, the "go" signal reappeared, and the animal was required to press the opposite panel (the previously unrewarded one) in order to receive another 0.03 cc of fluid. If the animal pressed the incorrect (just previously rewarded) panel when the "go" signal appeared, the trial terminated without producing the reward, and the animal had to wait out the delay period before it could try again. This procedure was a delayed correction technique since the animal was forced to make a correct response (i.e. alternate) before being rewarded. The monkey was not rewarded so long as it perseverated in pressing the wrong side. Each time the animal responded during the delay interval (premature response), the delay timer automatically reset to zero, and the animal had to wait at least the prescribed time before it could make a rewarded response, or an error (both mature responses) again. It must be noted that this procedure differed from the fixed interval (FI) schedule shown to be susceptible to drugs (Dews, 1955) in that with FI the animal ordinarily is given no cue that the interval has ended.

Training sessions were conducted daily and lasted 30 minutes for each animal. Stable performance levels were established before drug administrations were begun. Injection of all drugs was intramuscular into the posterior thigh muscles (hamstrings) and preceded testing by 15 minutes. Doses of LSD ranged from 0.005 to 0.2 mg/kg. Compari-

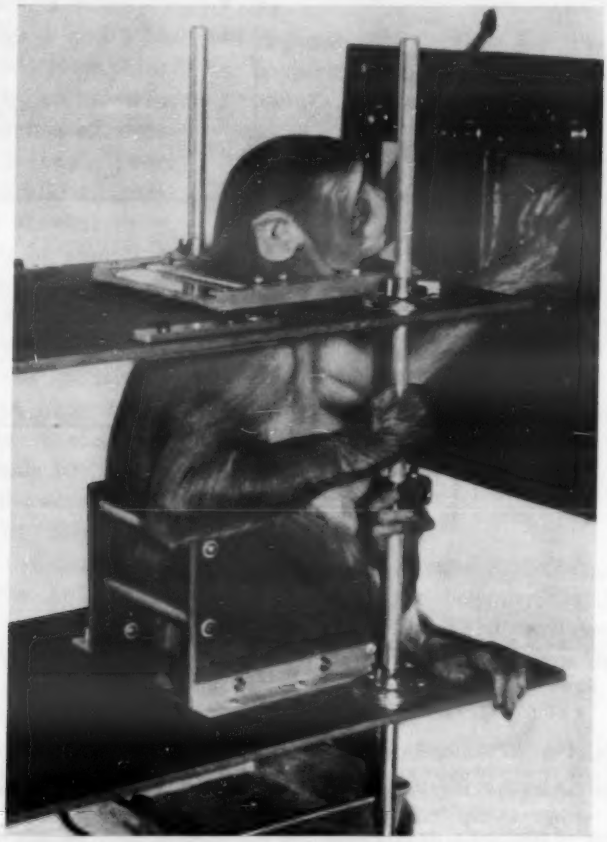

Fig. 1. Monkey pressing correct panel to receive liquid reward. When performing correctly, animal presses left and right panels alternately every 2 seconds

son with the effects produced by various doses of chlorpromazine, pentobarbital and amphetamine were made by administering these drugs to some of the animals several weeks after the termination of LSD.

Results

The depressant effects of LSD upon accuracy in the delayed alternation test is evident from Table 1. This shows both the accuracy and the total number of mature responses made by an animal during the half hour after receiving its first dose of LSD. Corresponding data are given

for the day prior to LSD — a representative control day. The four animals that received 0.025 mg/kg had at least a 30% fall in accuracy, whereas the scores of the four that received 0.005 mg/kg declined less than 21% of their control value on the previous day. The relative effect of the

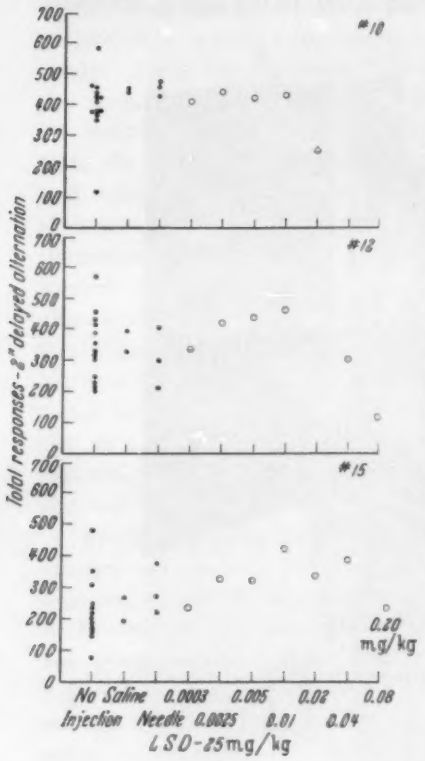

Fig. 2

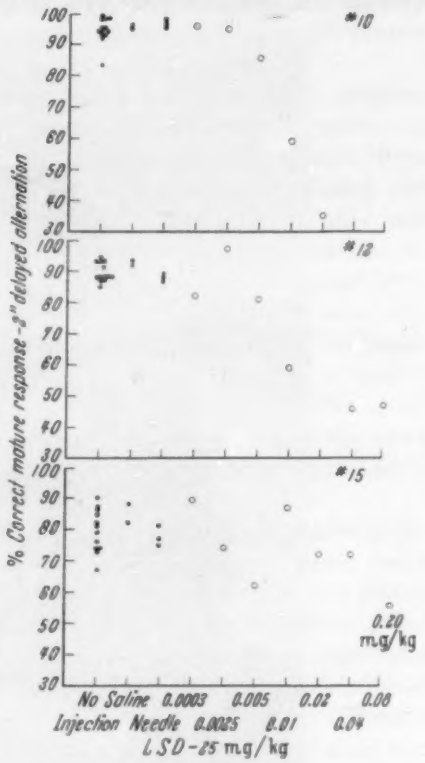

Fig. 3

Fig. 2. Dose-effect relationship, showing depression of pressing rate by LSD. The solid dots represent control values and the open dots results obtained with indicated doses of LSD-25. Each point is based upon a single day's experiment

Fig. 3. Dose-effect relationship, showing impairment of accuracy by LSD. The solid dots represent control values and the open dots results obtained with indicated doses of LSD-25. Each point is based upon a single day's experiment

two doses upon total number of responses was not as consistent as upon accuracy, but none of the animals receiving the small dose gave less than 100 mature responses in one half hour, whereas only one of the animals with the larger dose gave more than 100 presses (monkey 12 and, strangely enough, this was the animal with the biggest depression in accuracy).

Since there is no overlap between the control and experimental values, it is obvious that the differences for both percent correct and number

of responses are highly significant. The question of whether accuracy or rate was influenced more by LSD could not be answered from these data without instances in which one was affected and not the other. Consequently, some of the animals were given varying doses of the drug, so that dose-response relationships could be examined with respect to both accuracy and rate.

In Figs. 2 and 3 the dose-response relationships for three animals are plotted. Each point in Fig. 2 represents the total number of responses emitted by an animal in a 30 minute session while under the influence of the indicated dose. In Fig. 3 each point represents the accuracy achieved at that dose.

Table. 1. *Effect of First Dose of LSD on Accuracy and Rate of Delayed Alternation Performance*

Monkey	Day before LSD administration		Day of administration — LSD		
	Percent correct	No. of mature responses	Dose: in mg/kg	Percent correct	No. of mature responses
10	99	427	0.025	58	45
12	89	410	0.025	46	242
13	88	200	0.025	56	16
15	74	306	0.025	50	11
14	80	201	0.005	70	160
17	92	301	0.005	71	111
18	91	262	0.005	74	198
20	91	299	0.005	77	245

It can be seen that the three animals show marked differences in sensitivity to LSD with monkey 15 relatively tolerant of high doses. It is evident that for both animals 10 and 12, there is a dose of LSD capable of depressing accuracy without affecting pressing rate (0.01 and 0.02 mg/kg, respectively). For animal 15, this is not so clear because the drug had relatively less effect. Nevertheless, for animal 15 too, despite the great amount of overlap with the control values, there is a downward trend in accuracy with increasing dose and, if anything, an upward trend in rate.

It must be emphasized that these data, unlike those in Table 1, are derived from repeated injections of the same animal with different doses of LSD. There were always at least three days intervening between injections, but the possibility of interactions between injections and days was present. Subsequently, in order to see what interactions might occur if they were deliberately encouraged, animals were injected with a given dose of LSD for several consecutive days. The procedure was repeated with different doses separated by control periods. This experiment lasted approximately three months. The effects of such treatment upon accuracy of performance can be seen in Fig. 3.

Fig. 4 shows the daily performance of three animals with accuracy (percent correct) plotted against time (days). The steplike portions represent the effects of consecutive daily injections of LSD in the doses indicated on the graph. The steplike function is typical of the perfor-

mance of all of the animals and indicates the development of tolerance to the particular dose. It can be seen in Figs. 3A and B that a given dose (for example 0.02 mg/kg) given at the beginning of the experiment was more effective than the same dose administered later, after a degree of tolerance had been acquired. Original sensitivity to LSD was not regained even after drug-free periods of one to two weeks. In this case, the effect can be seen only with animals 10 and 12, and not with 15. For animal 15, none of the doses used produced a drop in accuracy significantly below control values, except 0.16 mg/kg. Even this large dose was effective only the first time it was given, as can be seen in Fig. 3C. Administration of the very same dose the following day did not prevent this animal from giving a very accurate performance.

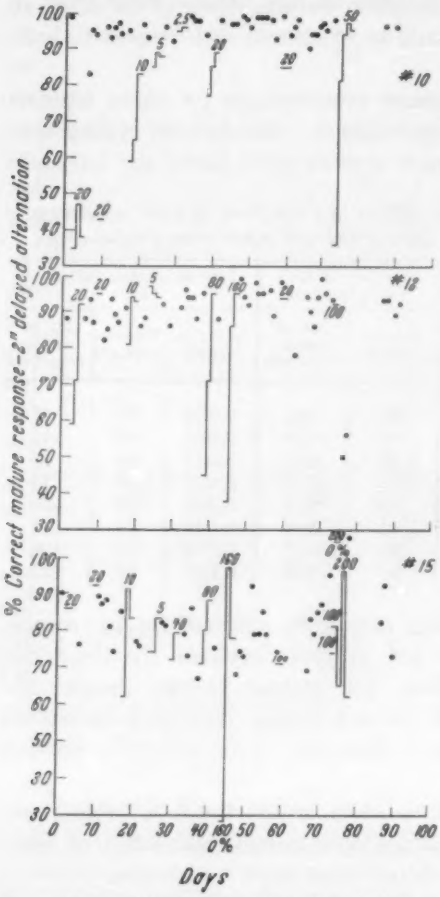

Fig. 4. Effect of repeated administration of LSD on accuracy in three animals. Note the rapid development of tolerance evidenced by the step-like improvement with a given dose, particularly prominent for animals 10 and 12. The small figures at the top of each step represent the dose of LSD, which was given for the period of consecutive days shown

Other drugs. In order to determine whether other drugs, which are known to affect the central nervous system, might produce a dissociation between accuracy and rate similar to that seen with LSD, selected doses of amphetamine, chlorpromazine, and pentobarbital were given to animals No. 10, No. 12, and No. 15. In Table 2, it is shown that in two of the animals, at least amphetamine was capable of markedly depressing the rate without particularly affecting accuracy. This is in contrast to LSD, which was capable of depressing accuracy without particularly affecting rate. The results in Table 3 with chlorpromazine and pentobarbital were gathered some months after the LSD experiments, and a

5 second instead of 2 second delay was used. Despite this change in procedure, some interesting contrasts with LSD emerged. Chlorpromazine in a dose of 0.5 mg/kg was capable of markedly depressing responding rate without influencing accuracy at all, quite the opposite effect of the lower dose of LSD. The effects of pentobarbital were more variable. In general, this appeared to depress rate, but in the doses used, its effect was much less marked than that of chlorpromazine and in one animal (No. 12) the number of responses actually increased. Monkey No. 15, in contrast to its resistance to LSD, was more susceptible than the other two monkeys to pentobarbital and was actually anesthetized by the larger dose (20.0 mg/kg).

Table 2. *Effect of Amphetamine Upon Delayed Alternation Performance (2 sec Delay)*

Monkey	Control			Amphetamine	
	Percent correct	No. of Mature responses	Dose mg/kg	Percent correct	No. of Mature responses
10	96	408	0.1	98	446
			0.25	94	231
			0.5	—	2
12	83	382	0.1	85	442
			0.25	79	112
			0.5	76	33
15	81	417	0.1	77	427
			0.25	85	180
			0.5	74	292

Table 3. *Effect of Chlorpromazine and Pentobarbital Upon Delayed Alternation Performance (5 sec Delay)*

Monkey	Control		Chlorpromazine			Pentobarbital		
	Percent correct	No. of Mature responses	Dose mg/kg	Percent correct	No. of Mature responses	Dose mg/kg	Percent correct	No. of Mature responses
10	97	246	0.5	96	25	5.0	99	171
						10.0	96	213
						20.0	83	145
12	90	192	0.5	96	27	5.0	88	225
						10.0	95	204
						20.0	89	232
15	79	266	0.5	79	198	5.0	67	239
						10.0	70	248
						20.0	66	3

Discussion

Of the drugs which were used in this experiment only LSD seemed capable of affecting the accuracy of performance of the delayed alternation task without affecting the rate. In this respect, the results which were obtained following LSD administration resemble those seen in monkeys with frontal lobe lesions. However, it has not yet been determined whether the automatic apparatus, which was employed in the

present study, tests the same functions as manual testing methods which have been used in ablation studies. With the present automatic apparatus, it is possible for an animal to make premature responses since the manipulanda are available during the intertrial interval in contrast to those in the traditional Wisconsin General Test Apparatus. The reason for the relative increase in errors with LSD is still a mystery, but appears to be dissociable from factors responsible for a decrease in rate of responding. As WIKLER (1957) has noted, "In spite of the many difficulties encountered in attempting to reconcile the data on the neurophysiological mechanisms of action of 'psychosomimetic' agents, it appears quite clear that they are capable of altering profoundly the patterns of corticipetal afferent impulses originating in the periphery, in a manner quite different from those of other drugs ... That such alteration of sensory input may produce marked changes in 'mental' and 'motor' output has been suggested by several investigators ... and this is supported by evidence independent of pharmacological data ..." It is possible that under the influence of LSD individuals are rendered more susceptible to the distracting effects of both internal and external stimuli, as has been suggested by BRADLEY and ELKES (1957) and are less capable of handling a delay type of situation.

The results of this experiment demonstrate that tolerance to LSD develops rapidly and, in addition, may persist for long periods (at least two weeks) when no drug is given. The rapid development of tolerance to LSD has previously been noted in rabbits and humans (GOGERTY and DILLE, 1956; ISBELL, FRASER, WIKLER, and BELLEVILLE, 1955) and is a major source of variance when repeated doses of this drug are used in the same subject.

The cumulative effects of LSD and the variability in susceptibility make it difficult to establish threshold doses which will uniformly and repeatedly affect delayed alternation performance in different animals. In general, it would appear from the present study that a previously untreated animal will respond to an initial dose of LSD with impaired accuracy, and a moderately depressed rate of responding, but that on immediately subsequent days, the same dose will be less effective in modifying its responses.

It has been shown that accuracy of choice of equally appearing alternatives is a function which is much less susceptible to generalized physiological influences, such as motivational changes, than is the rate of responding (MEYER, 1951; and MILES, 1959). The rather unexpected finding is that LSD can, under certain circumstances, depress accuracy on a delayed alternation test without affecting rate suggests that, under these conditions, it must work primarily on some nervous mechanism other than those controlling the simple instrumental response of pressing for water.

Summary and Conclusions

Monkeys were found to show impairment in performing the delayed alternation problem following injections of LSD in initial doses as low as 0.005 mg/kg. The effect of this drug was primarily reflected in a decrease in accuracy of response, although increasingly large doses tended also to depress the rate of performance. Tolerance to the effects of LSD rapidly developed and persisted for several days, at least.

Unlike LSD, amphetamine, pentobarbital and chlorpromazine either did not produce changes in accuracy, or when they did, these changes were relatively small compared to the effects of these drugs on the pressing rate. The possibility is suggested that the partial similarity of the effects of LSD to those obtained in monkeys following bifrontal lesions may reflect the operation of some common factor.

Acknowledgements. The lysergic acid diethylamide used in this experiment was generously supplied by Dr. RUDOLF BIRCHER of Sandoz Pharmaceuticals, Hanover, New Jersey.

The chlorpromazine used in this experiment was generously supplied by Dr. LEONARD COOK of Smith, Kline and French Laboratories, Philadelphia.

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The Effect of Several Psychotomimetic Drugs on Human Serum Cholinesterase

By

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With 4 Figures in the Text

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Several drugs reported to cause psychotomimetic symptoms in man inhibit human serum cholinesterase. The most potent of the psychotomimetic drugs, lysergic acid diethylamide (LSD), at 10^{-6} M produces 50 per cent inhibition of hydrolysis of several aliphatic substrates (THOMPSON et al. 1955). Bromlysergic acid (BOL), ineffective as a psychotomimetic agent (CERLETTI and ROTHLIN 1955) inhibits serum cholinesterase almost as effectively as does LSD with benzoylcholine (BzCh) and acetylcholine (ACh) as substrates, measured manometrically (ZEHNDER and CERLETTI 1956). FRIED and ANTAPOL (1957) confirmed this inhibitory effect of LSD but reported that lower concentrations (10^{-9} M) activated human serum cholinesterase, as measured manometrically with ACh as substrate.

Among the drugs considered by EVARTS (1957) to meet his criteria as psychotomimetic agents: diisopropylfluorophosphonate (DFP) (MASUR and BODANSKY 1946) and tetraethylpyrophosphate (TEPP) (MANGUN and DUBOIS 1947) are among the most potent serum cholinesterase inhibitors known; nalorphine inhibits horse (BLOHM and WILLMORE 1951) but not dog (YOUNG et al. 1955) serum cholinesterase, while the only report of its effect on human serum cholinesterase is of activation (FOLDES et al. 1957a). Mescaline and adrenochrome are weak inhibitors of human serum cholinesterase (GOLDENBERG and GOLDENBERG 1956) when ACh is used as substrate. No reports of the effects of Harmine and of tetrahydrocannabinol on serum cholinesterase have been noted.

In most of these studies ACh was used as substrate. BzCh hydrolysis by serum cholinesterase differs from ACh hydrolysis both kinetically (AUGUSTINSSON 1950; KALOW et al. 1956) and in sensitivity to various inhibitors (FOLDES et al. 1957b). The theoretical advantages of testing the effect of inhibitors on dilute (5×10^{-5} M) solution of BzCh have been discussed by KALOW et al. (1956). Also, the occurrence of a genetically determined atypical form of human serum cholinesterase which differs

from normal in its affinity for certain inhibitors and substrates has been demonstrated (KALOW and GENEST 1957; KALOW and DAVIES 1995).

This study was designed primarily to provide directly comparable data on the cholinesterase inhibiting properties of several psychotomimetic drugs obtained under similar standardized conditions. Both typical and atypical enzymes have been tested in so far as the available supply of the latter permitted[1].

From the psychotomimetic agents listed by EVARTS (1957), tetraethylpyrophosphate was not available for study, adrenochrome was excluded because its extreme instability makes it unsatisfactory for inclusion (GREEN and RICHTER 1937), and tetrahydrocannabinol was excluded because of its limited solubility. Three recently introduced psychotomimetics have been included: 2-diethylaminoethyl cyclopentyl (2 thienyl) glycolate hydrochloride, designated WIN 2299 (PENNES and HOCH 1957), N,N-dimethyltryptamine (ST. SZARA 1956; SAI-HALÁSZ et al. 1958), and Psilocybin (HOFMANN et al. 1958). Additional drugs tested were: D-amphetamine, because of recent reports of psychoses occurring in connection with its use (CONNELL 1958; ASKEVOLD 1959); cocaine, because of the occurrence in addicts of hallucinations and paranoid thinking (GOODMAN and GILMAN 1955); BOL, chlorpromazine, and meprobamate, for purposes of comparison.

Methods

Cholinesterase inhibition was measured by the method of KALOW and GENEST (1957), modified by the substitution of appropriate inhibitors in various concentrations. Determinations of hydrolytic activity, with and without inhibitor, with BzCh (5×10^{-5} M) as substrate, were made by measuring the change in absorbance at 240 mμ in a Beckman DU spectrophotometer equipped with thermospacers. All determinations were made between 25° C and 27° C and the value corrected to 26° C by the formula of KALOW and GENEST (1957). 0.15 M Phosphate buffer at p_H 7.4 was used as solvent throughout. All values reported represent duplicate determinations.

[1] The author is grateful to the following people for generous supplies of drugs: Mr. SIDNEY GIMPEL, Sandoz Pharmaceuticals, Hanover, N.J. (BOL); Dr. HARRIS ISBELL, NIMH Addiction Research Center, Lexington, Ky. (Psilocybin); Dr. M. L. TAINTER, Sterling-Winthrop Research Institute, Rensselaer, N.Y. (WIN 2299); Mr. J. A. WOLFF, Merck & Co., Rahway, N. J. (DFP); Dr. H. SHEPPARD, Ciba Pharmaceutical Products, Summit, N.J. (Dibucaine); Dr. EMILY H. WOLF, Wallace Laboratories, New Brunswick, N.J. (Meprobamate); Dr. M. K. TUCKER, Smith, Klinie and French Laboratories, 1500 Spring Garden Street, Philadelphia, Pa. (Chlorpromazine).

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Whole human blood serum diluted to a final concentration of 1:200 was used as a source of enzyme. The blood with typical cholinesterase was drawn from young adult males free of manifest disease. The blood was allowed to stand at room temperature for 4—6 hours; then it was centrifuged and the serum separated and refrigerated until time of use. Inhibition characteristics remained constant under these circumstances for at least several months.

Atypical enzyme was obtained from patients who had a history of atypical response to succinylcholine. The blood was treated in the same manner as for the typical enzyme.

Except with DFP all inhibitor concentrations indicate the concentration in the final reaction mixture. Among the drugs tested in this study, only DFP required preincubation with the enzyme prior to addition of substrate in order to achieve maximum inhibition. All other drugs were added to the enzyme simultaneously with the substrate. DFP was added to separate samples of diluted serum, both at the time the substrate was added and at measured intervals prior to the addition of substrate. The concentrations of DFP represent the inhibitor in the serum-inhibitor mixture rather than in the final reaction mixture.

For some of the inhibitors the concentration which could be tested effectively in our system was limited by the ultraviolet absorbance of the inhibitor. No attempt was made to test inhibitor concentrations with initial absorbances above 1.000.

Results

Blood samples from two subjects with atypical cholinesterase have been studied in this laboratory. Both were referred because of prolonged apnea after injections of succinylcholine.

Fig. 1 shows the inhibitions obtained with various concentrations of dibucaine at 26° C, p_H 7.4, in M/15 phosphate buffer with BzCh as substrate using the two types of enzymes. Defining the dibucaine number (DN) as the percentage inhibition produced by 10^{-5} M dibucaine under the specified conditions (KALOW and GENEST 1957), the typical enzyme has a DN of 80; the atypical enzyme a DN of 18.

[Serum samples from seven subjects with intermediate dibucaine numbers ranging from 55 to 65 have been examined in this laboratory. Since these sera apparently contain a mixture of typical and atypical enzyme (KALOW and DAVIES 1959) they have not been included in the present study.]

LSD 25. Fig. 2 shows the percentage inhibition obtained with various concentrations of LSD 25 using sera from two subjects with typical cholinesterase and from one subject with atypical cholinesterase.

With LSD 25 as inhibitor the negative logarithm of the molar concentration necessary to produce 50 per cent inhibition (pI_{50}) of typical cholinesterase is 6.55; the pI_{50} for atypical cholinesterase is 6.20.

The activation reported to occur with lower concentrations of LSD (FRIED and ANTAPOL 1957) was not observed in this system with concentrations as low as 10^{-10} M.

In addition to the above data, the inhibition produced by a single concentration of LSD 25 ($10^{-6.5}$ M) has been measured individually using serum obtained from 16 young males who were free of manifest disease. All these subjects had typical cholinesterase with DN's ranging from 76 to 83. The per cent inhibition produced by $10^{-6.5}$ M LSD ranged from 49 per cent to 58 per cent (mean 54.8 per cent).

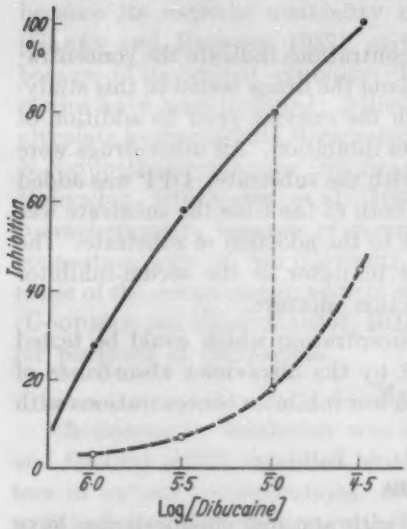

Fig. 1. The inhibition of two types of human serum cholinesterase produced by dibucaine. ●—— Typical; ○———○ Atypical

BOL 148. Fig. 3 shows the percentage inhibition obtained with various concentrations of BOL 148, using sera from one subject with typical cholinesterase and from two patients with atypical cholinesterase. With BOL 148, the pI_{50} for typical cholinesterase is 6.15; the pI_{50} for atypical cholinesterase is 5.70.

Mescaline. Concentrations of mescaline up to 10^{-4} M produced no measurable inhibition of typical cholinesterase. The effect of higher concentrations could not be measured in this system because of the absorbance by mescaline.

N-allylnormorphine. N-allylnormorphine, 10^{-4} M, produced 38 per cent inhibition of typical cholinesterase and six per cent inhibition of atypical cholinesterase. At $10^{-4.5}$ M it inhibited typical cholinesterase 15 per cent but produced no measurable inhibition of atypical cholinesterase. No activation of BzCh hydrolysis was noted at any of the concentrations tested.

Dimethyltryptamine. Inhibition rates for the typical and atypical enzyme, respectively, were 50 per cent and 16 per cent at 10^{-4} M, 25 per cent and 7 per cent at $10^{-4.5}$ M, 11 per cent and 6 per cent at 10^{-5} M, 5 percent and 4 per cent at $10^{-5.5}$ M, and 0 per cent for both enzymes at 10^{-6} M.

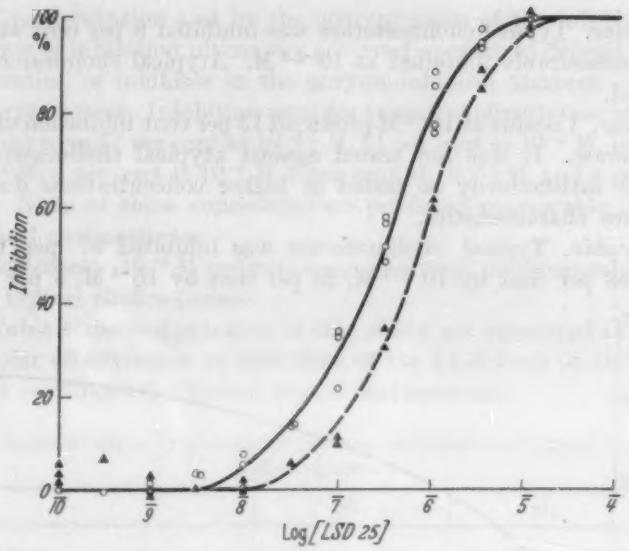

Fig. 2. The inhibition of two types of human serum cholinesterase produced by LSD.
○——○ Typical cholinesterase; ▲——▲ Atypical cholinesterase

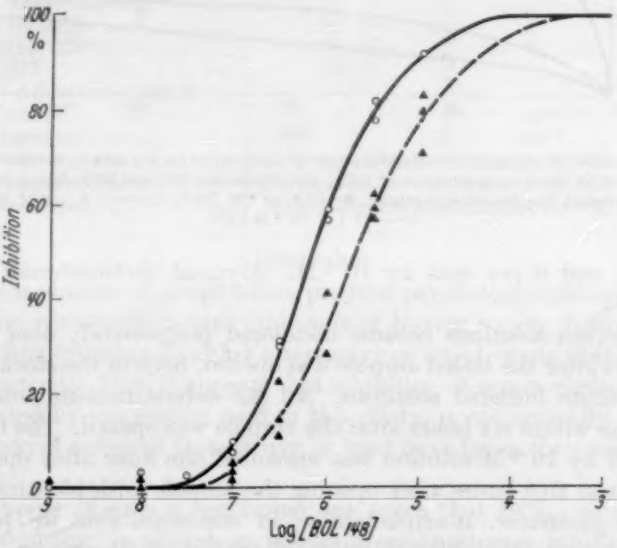

Fig. 3. The inhibition of two types of human serum cholinesterase produced by Brom-LSD.
○——○ Typical cholinesterase; ▲——▲ Atypical cholinesterase

WIN 2299. Typical cholinesterase was inhibited 54 per cent at $10^{-4.5}$ M and 34 per cent at 10^{-5} M. Atypical enzyme was not inhibited measurably at either of these concentrations.

Harmine. Typical cholinesterase was inhibited 9 per cent at 10^{-4} M but not measurably inhibited at $10^{-4.5}$ M. Atypical cholinesterase was not tested.

Cocaine. Cocaine at 10^{-5} M produced 13 per cent inhibition of typical cholinesterase. It was not tested against atypical cholinesterase and could not satisfactorily be tested at higher concentrations due to its absorbance characteristics.

Psilocybin. Typical cholinesterase was inhibited 97 per cent by 10^{-4} M, 68 per cent by $10^{-4.5}$ M, 25 per cent by 10^{-5} M, 5 per cent by

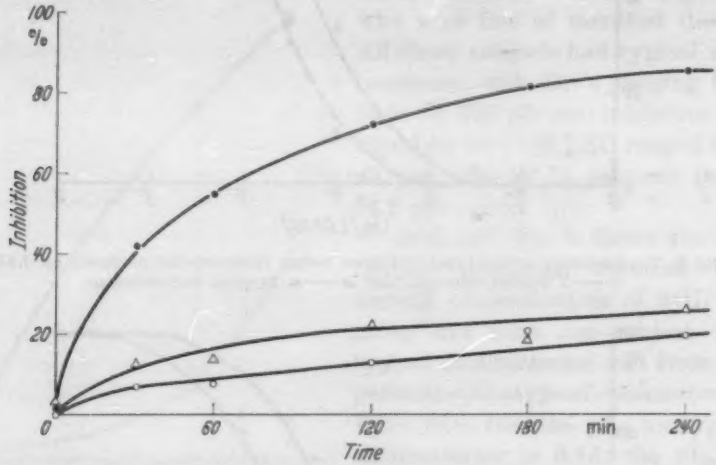

Fig. 4. The effect of preincubation time of serum with inhibitor on the serum cholinesterase inhibition produced by three concentrations of DFP. Concentrations indicate DFP during preincubation rather than in final reaction mixture. ●——● 10^{-4} M DFP; △——△ 2×10^{-5} M DFP; ○——○ 1×10^{-5} M DFP

$10^{-5.5}$ M, and 0 per cent by 10^{-6} M. Atypical cholinesterase was not tested.

Psilocybin solutions became discolored progressively over the first few hours after the sealed ampule was opened, both in the stock solution and in dilute buffered solutions. All the determinations noted above were done within six hours after the ampule was opened. The inhibition produced by 10^{-5} M solution was measured one hour after opening the ampule and five hours after opening the ampule with identical results.

D-*Amphetamine.* D-amphetamine in concentrations up to 10^{-3} M produced no measurable inhibition of typical cholinesterase.

Diisopropylfluorophosphonate (DFP). Fig. 4 shows the effect of DFP on typical cholinesterase with various times of preincubation prior to adding substrate. Addition of DFP to serum simultaneously with substrate resulted in no measurable inhibition. The inhibition observed after preincubation with DFP appears initially to be limited both by the

time of preincubation and by the concentration of the inhibitor, while the degree of inhibition ultimately achieved appears to depend upon the concentration of inhibitor in the enzyme-inhibitor mixture.

Chlorpromazine. Inhibition rates for typical cholinesterase with chlorpromazine were 77 per cent at $10^{-4.5}$ M, 51 per cent at 10^{-5} M, 19 per cent at $10^{-5.5}$ M, 4 per cent at 10^{-6} M, 2 per cent at $10^{-6.5}$ M, and 0 per cent at 10^{-7} M. None of these concentrations produced measurable inhibition of atypical cholinesterase.

Meprobamate. 10^{-4} M meprobamate produced no measurable inhibition of typical cholinesterase.

In Table 1 the drugs tested in this study are tabulated in order of their molar effectiveness as inhibitors of the hydrolysis of BzCh under specified conditions by human serum cholinesterase.

Table 1. *Approximate order of molar effectiveness as inhibitors of typical human serum cholinesterase*

	pI_{50}	pI_{10}
DFP	see figure 4	
LSD	$10^{-6.5}$	
BOL 148	$10^{-6.0}$	
Chlorpromazine	$10^{-5.9}$	
Psilocybin	$10^{-4.6\pm}$	
WIN 2299	$10^{-4.5\pm}$	
DMT	$10^{-4.0}$	
N-Allylnormorphine	$10^{-3.8}$	
Cocaine		10^{-5}
Harmine		10^{-4}
Meprobamate	No measurable inhibition at 10^{-4} M	
D-Amphetamine	No measurable inhibition at 10^{-3} M	
Mescaline	No measurable inhibition at 10^{-4} M	

Discussion

While a number of drugs which produce psychotomimetic symptoms in man are reasonably potent inhibitors of human serum cholinesterase, this does not appear to be either a necessary or an adequate characteristic for such activity. Thus it appears that inhibition of serum cholinesterase, as evaluated by the system used in this study, is not critically involved in the mode of action of these drugs, at least in so far as they are psychotomimetic.

In support of such a conclusion one notes that BOL, which is not psychotomimetic, is almost as potent a cholinesterase inhibitor as is LSD (see also ZEHNDER and CERLETTI 1956), while chlorpromazine, which is not psychotomimetic and may even antagonize the effects of LSD (HOCH 1955; ISBELL and LOGAN 1957), is itself a cholinesterase inhibitor exceeding in molar effectiveness some of the psychotomimetic drugs. On the other hand, mescaline, a less potent but specific psychotomimetic

agent (KOELLE 1958; BEECHER 1958), has little if any effect on human serum cholinesterase.

To any such generalization, however, certain distinct reservations must be added. One such reservation concerns choice of substrate and assay system. For instance, other investigators using other assay systems (THOMPSON et al. 1955; ZEHNDER and CERLETTI 1956; GOLDENBERG 1956) report 50 per cent inhibition with concentrations of LSD 10 to 20 times as great as that producing 50 per cent inhibition in this assay system (BzCh 5×10^{-5} M; p_H 7.4 PO_4 buffer; 26° C). Similar differences are noted also with BOL and in the GOLDENBERG's study (1956) with chlorpromazine. Since the physiologic substrate of serum cholinesterase is not known, one can only conjecture which, if either, more accurately reflects the inhibition that obtains *in vivo*.

Also, caution is necessary in attempting to extend these observations to the cholinesterase of other tissues, particularly of brain. Although THOMPSON et al. (1955) obtained similar inhibition rates for brain and serum cholinesterase with LSD, as did ORD and THOMPSON (1952) with several other inhibitors, it is still not certain that the enzymes occurring in the two tissues are identical. Several investigators, in fact, have produced evidence that brain contains several hydrolytic enzymes (MYERS 1956; PEPLER and PEARSE 1957) closely resembling "cholinesterase" in substrate specificities but differing somewhat both in specificities for substrates and inhibitors. Accordingly, the data reported here cannot be expected to apply rigidly to the 'cholinesterases' of the nervous system.

In general, our findings have confirmed those of KALOW and his co-workers (KALOW and GENEST 1957; KALOW and STARON 1957) regarding the occurrence of an atypical form of human serum cholinesterase, differing from normal in its sensitivity to a number of inhibitors. Our data show, however, that while the two forms differ in sensitivity to LSD and BOL, the degree of difference does not conform to the mathematical relationship described for inhibitors believed to react with the anionic site of the enzyme (KALOW and DAVIES 1959). Rather, there is a smaller difference than would be anticipated on the basis of an anionic site reaction. On the other hand, DFP and TEPP, believed to react with the esteratic site, affect both enzymes equally (KALOW and DAVIES 1959). This may indicate that LSD and BOL are reacting with both the esteratic and the anionic sites of the enzyme. Whatever the basis for the difference, however, neither LSD nor BOL fits into any of the three classes of inhibitors defined by KALOW and DAVIES (1959).

Summary

The effects of a number of drugs of psychiatric interest on human serum cholinesterase are reported.

The reported occurrence of an atypical form of human serum cholinesterase, differing in sensitivity to some inhibitors, has been confirmed.

LSD and BOL, both potent inhibitors of human serum cholinesterase, do not fit into any of the three groups of inhibitors defined by their relative effectiveness as inhibitors of typical and atypical human serum cholinesterase.

Some of the limitations of these findings are discussed.

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Prolonged Reactions to Mescaline* **

A Report of Two Cases

By

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Introduction

The increasing use of psychotomimetic drugs in investigations and for treatment makes probable the occurrence of larger numbers of prolonged reactions to these drugs unless we can learn more about such reactions so as to reduce their incidence. Reports of such prolonged reactions circulate among investigators by word of mouth and through correspondence, but few have been published.

MORSELLI (1936) published a moving autobiographical account of his experiences after ingesting 750 mg. of mescaline. He was alone at first in his apartment and experienced a severe reaction with visual hallucinations, depersonalization and fear of complete mental disorganization. He subsequently, and perhaps at the time, compared his state to catatonia. With great effort he succeeded in reaching his hospital where the hallucinations and disorganization of thought processes gradually wore off, having lasted altogether 16 hours. For more than two months after this, however, MORSELLI suffered from a belief that a man in one of the pictures in his apartment (which had figured in his hallucinations at the height of the mescaline effect) was living and haunting him with hostile intentions. No sensory abnormalities occurred during this phase. MORSELLI suffered less from his impression of being haunted if he left the apartment, but removing the portrait from the apartment did not reduce the experience. When he re-entered or even thought of returning to his apartment, the impression would revive. At times his anxiety with regard to this experience drove him to sleep away from his apartment. MORSELLI preserved throughout this ordeal an insight into the relationship between this vivid and frightening experience and his inge-

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stion of mescaline; this did not help him. After about two months, the symptom gradually cleared completely. There were no other sequelae of the ingestion of the drug.

HOFFER, et al. (1954) reported one instance of a reaction to adrenochrome lasting four days and OSMOND (1955) a reaction lasting four months. COOPER (1955) reported the recrudescence of symptoms of intoxication with LSD-25 weeks and months after the drug had been taken. His report mentioned the recurrence (although not necessarily in one patient) of almost all the symptoms which ordinarily occur during intoxication with LSD-25. SAVAGE (1957) reported an instance of suicide in a patient who had taken LSD-25. MARTIN (1957) and SANDISON and WHITELAW (1957) reported the recurrence of symptoms in persons taking LSD-25 after an interval of normality up to as long as two years later. SANDISON and WHITELAW (1957) also reported an instance of an unsuccessful attempt at suicide on the part of one of their patients when under the influence of LSD-25. EISNER and COHEN (1958) reported an instance of prolonged reaction to LSD characterized by paranoid thinking. ELKES, et al. (1955) have emphasized the dangers of severe and prolonged reactions to LSD-25, although they do not describe specific instances in their brief note. HARLEY-MASON, et al. (1958) have published notes on three delayed reactions to mescaline. Some of these reactions recurred after an interval of recovery.

The following two cases add to the information on this subject. The first case also illustrates the combination of the effects of mescaline with factors within the subject's personality in the production of a brief psychosis indistinguishable from a schizophrenic reaction. These two prolonged reactions were the only ones of their kind occurring in 47 experiments with 25 subjects, the main results of which have been reported elsewhere (STEVENSON and SANCHEZ 1957, STEVENSON and MOKRASCH 1958, MOKRASCH and STEVENSON 1959). In these experiments the mescaline was administered between 7 and 8 a. m. on the day of the experiment. With the exception of these two cases, all the subjects reported that they felt entirely well in 24 hours or less. Most seemed to have recovered completely by the evening of the day of the experiment.

Subjects for these experiments were obtained by calling for volunteers from the staff and medical students of the Louisiana State University School of Medicine. Volunteers were interviewed before the experiments. Subjects with known physical illnesses or apparent marked manifest anxiety were excluded from the experiments. Most of the subjects were given psychological tests by a clinical psychologist. For investigative purposes, the psychologist did not show his data to the other investigators until after the subjects had taken mescaline; and he was not told

of the reactions of the subjects to mescaline until after he had tested the subjects and evaluated his data. Psychological test data were accordingly not used to exclude any subjects from the experiments.

Case reports

Case No. 1, Mr. J. B., was a medical student, 24 years of age, in good general health. He received 400 mg. mescaline orally on two occasions at 7 a.m. On the first occasion, he experienced some elevation of mood, slight changes in visual imagery and marked feelings of unreality. Between four and five hours after ingesting the mescaline, he was given 24 g of sodium succinate intravenously, following which his symptoms of mescaline intoxication diminished markedly (STEVENSON and SANCHEZ, 1957). However, later in the afternoon about ten hours after taking the mescaline (5 p.m.) the symptoms again returned and another 6 g of sodium succinate were administered intravenously with further improvement. Eleven hours after taking the mescaline the subject was taken home, and at that time seemed to have largely, although not entirely, recovered from the effects of the drug. Two hours later (8 p.m.) he reported still experiencing some effects of the drug, but these were diminishing and the following morning he felt entirely well.

Two weeks later the subject participated in a second experiment, on which occasion he did not receive sodium succinate as an antidote to the effect of the mescaline given in the same dosage. On this occasion feelings of unreality were a little stronger and elation less marked. He reported that the second experience was more pleasant than the first one. He believed that the experiences were similar qualitatively but that the second was quantitatively less marked. The investigators observing him believed (contrary to his own opinion) that his second reaction was stronger than his first. Both of this subject's reactions to mescaline were judged by the investigators (who used a rating scale) to have been more severe than that of most of the other subjects of this series of experiments.

Eleven hours after taking the mescaline (6 p. m.) the subject's condition had improved sufficiently so that it was judged safe to take him home. Two hours later, in a telephone conversation, the subject reported himself to be having a relapse and said that things again seemed unreal. This time he was depressed and perplexed. Two hours later (10 p.m.) these symptoms had diminished. The following morning the subject felt entirely well and went to work. He worked hard for twelve hours at a stretch. That evening he noticed a return of feelings of unreality. The next morning he found himself apathetic and failed to do his work properly. He moved slowly, seemed to be in a daze, forgot to carry out assignments or, if he did them, failed to record them properly. His

apathy and inactivity were noticed by his wife who was also a student; she was able to perform some of his duties. At the same time, she noticed that the subject appeared indifferent to the accumulation of his unfinished work.

On the evening of the second day after the mescaline, the subject reported the prolongation of his symptoms and the investigator examined him at his home. He was then concerned about his continuing feelings of unreality and believed that he had become psychotic. He was slow in his movements and appeared apathetic; to the extent that he showed any affect, he was depressed. He was reassured and given a sedative to aid his sleeping, but slept poorly that night and was awakened by an unpleasant dream. The next day he worked more effectively and reported an improvement in his symptoms. The following day he again reported himself to be feeling somewhat better. However, an examination on the day after this showed that his symptoms were in fact continuing and had become somewhat worse. He was troubled by feelings of unreality, by apathy, inertia, and inability to concentrate. He also had disturbances of sensation, believing that his face was anesthetic when he shaved. He talked slowly with little emotional expression, although occasionally smiling and at other times looking angry. He continued preoccupied with his feelings of unreality and exhibited a diffuse hypochondriasis. At one moment he said he believed that he had a serious mental illness; he requested an injection to make him better. Then he was sure he had syphilis; he wanted a blood test to exclude paresis and said that two years previously he had pricked his skin with a needle used on a syphilitic patient.

On this occasion, it was thought that the subject's feelings of unreality might derive from a wish to withdraw from unpleasant circumstances in his life. So the investigator, now acting as a therapist, explored this possibility with the subject, now a patient, and learned that he had a lifelong habit of procrastinating the performance of unpleasant tasks and indulging in day-dreaming when under stresses. When as a child his mother would ask him to perform various household chores he would drag these out for hours when he might have accomplished them in a few minutes. He recalled that during the period when he was supposed to be doing these tasks he would day-dream and on these occasions frequently had feelings of unreality similar to those he experienced during and after taking mescaline. The interview further showed that the patient's behavior towards his wife repeated that towards his mother. He rebelled against his wife's suggestions or coaxing; he would sleep late in the morning so that he would be late for work, and if she woke him, he would accuse her of nagging. The subject and his wife then confided their suspicion that she was pregnant. This pregnancy she much desired,

but he was opposed to it both on grounds of financial difficulties and his own doubts about his readiness to be a father.

As these facts emerged, the therapist pointed out to the subject the similarity between his behavior towards his mother and that towards his wife, and the temptation he might be under to withdraw from his current stresses into the state of unreality and apathy which he was exhibiting. He acknowledged that the effects of the mescaline had "provided me with a model for solving my problems".

The day after this interview, the subject reported himself much better and his claimed improvement for the first time was also apparent to the therapist. His affect was freer and he was able to laugh and smile more easily. He attributed some of his improvement to the therapist's interpretative statements that his behavior towards his wife resembled that of a boy towards his mother. The patient then reported three dreams which he had recently had and said that they recapitulated in reverse order some stages of his growth. In the first dream, he was pursuing a sexually exciting woman. In the second, he was with a woman who was older than he, apparently a mother figure, but walked away from her with guilty feelings. In the third dream, he was with some adolescent boys who were singing and he was moved by their singing and felt close to them and loath to leave. He said the second dream referred to his reluctance to give up his mother in order to marry his wife and the third dream referred to his reluctance to give up adolescence.

During the following four days, the subject's feelings of unreality and his apathy continued at the same level, but did not completely disappear until the eleventh day after the experimental day. During the last few days of the continuing symptoms he became quite discouraged about their persistence and found himself wishing to die rather than to go on in that way. The final clearing occurred rather suddenly overnight, in that he went to bed still feeling the symptom of unreality which had been his chief complaint and awoke the next morning completely clear.

The subject graduated from medical school and then worked as an intern and subsequently as a resident in a university hospital. He continued to be extremely passive and unambitious, exhibiting to a lesser degree the apathy which he demonstrated so markedly for the eleven days after taking the mescaline. Eventually, the chief of his service urged him to seek psychotherapy which he did approximately two years after the mescaline experience. The psychotherapist, who saw him ten times, has communicated to us some of his observations. The subject was vague about the need for psychotherapy and put the responsibility for this on the chief of service who had recommended it. He was able, however, to talk about his forgetfulness and negligence at work. He complained of difficulty in concentrating and considerable tendencies to

irrelevant thinking. The psychotherapist learned from the subject that he had been markedly dependent on his mother. He also confirmed the impression mentioned above that the subject was extremely dependent upon his wife. The subject almost always arrived late for his interviews which behavior was one manifestation of his difficulty in organizing his use of time.

Colleagues of the investigators and of the subject also commented on his inappropriate lack of anxiety. For example, when hunting he was careless of his own personal safety and that of others. One day, a little more than two years after the experiments described, he went hunting in an unsafe boat which leaked and he then rashly jumped out of the boat into water which he believed shallow, but which was in fact quite deep, and he drowned in this water.

A clinical psychologist (T.W.R.) administered several psychological tests to this patient two months after the mescaline experiments. These were the Rorschach Test, Human Figure Drawing Test and Thematic Apperception Test. His interpretation of the test data included the following: "The subject was highly deviant (from other subjects of this series) in all variables ... He was considered severely neurotic (rather than psychotic) in his hypersensitivity, his excellent touch with reality and his need to be accepted and not rejected. Anxiety was prominent. There seemed not to be a nuclear psychotic way of handling stress, but rather there was a strong sensitivity to the possibility of failure."

Case No. 2, E. H. was a 22 year old female medical student who took 400 mg of mescaline orally at 7 a.m. on the day of the experiment. During the period of presumed maximal intoxication she lay on a bed quietly and communicated little with the investigators. Her outer behavior changed less than did her apparent thought processes which seemed to become quite disorganized. Her reaction seemed characterized chiefly by impoverishment of thought content; she complained of being unable to think or to think clearly. She showed impairment of her capacity to perform abstractions. She had moderate alterations in her perceptions with visual illusions and hallucinations. She developed considerable anxiety. She became suspicious of the observers so that when they proposed to give her an antidote she resolutely refused. Subjectively, her experience was most unpleasant and her complaints were stronger than those of most of the other subjects. She said, "I didn't have any thoughts. I couldn't say what I wanted to." The investigators judged her reaction at the period of maximal severity to have been more severe than those of most of the other subjects.

The symptoms abated somewhat between eight and ten hours after the injection of the drug and the subject returned to her room. However, that evening she continued to be anxious and unusually dependent

upon her roommate whom she did not wish to leave her. The following morning, her reaction continued with impaired attention and reduced emotional expression. Her thoughts then were somewhat speeded up rather than slowed as they had been during the height of the intoxication. Her suspiciousness remained. When she was offered some sedative pills she accepted them, but told her roommate she had no intention of using them. She attributed a special meaning to the fact that she was given one kind of sedative rather than another which she had expected. She wanted very much to leave the city for her home in a neighboring town and that afternoon did so. She later reported that for the rest of that day she had been unable to tell whether mosquitoes were real or imaginary. For several succeeding days she heard ringing bells, apparently an hallucinatory experience. After this, she recovered entirely. She refused, however, to participate in any further experiments of this kind.

Anamnestic and follow-up data of the kind available for the preceding subject were not available for this subject. She had been, however, tested by the clinical psychologist (T.W.R.) with the Rorschach and Human Figure Drawing Tests, which were administered two days before the mescaline experiment. The following statements are extracted from his interpretation of the data: "The subject seems to be basically introvertive, although she seems to devote considerable conscious effort toward being more outgoing and sociable. Social situations are threatening to her and she tends to see others not simply as people, but as people with the characteristics of another time (witches in MACBETH) or another, perhaps mythical world, or as silhouettes. Her figure drawings depict people who are frightened and her female figure shows a need to disguise sexuality. She was predicted to have the capacity to utilize a somewhat intellectualized paranoid defense (during mescaline) with little spontaneity showing through. Her deviations, as a whole, however, are not extreme."

Discussion

We still know rather little about why some persons taking psychotomimetic drugs have prolonged reactions while others do not. Some investigators, on the grounds that the liver plays an important part in detoxifying many drugs, probably including the amines, have avoided administering mescaline (and other hallucinogenic drugs) to persons with known liver disease. Prolonged reactions to mescaliine and adrenochrome (HOFFER, et al. 1954 and OSMOND 1955) have been attributed to liver disease. However, SMITH (1958) has reported that he observed no greater effects of LSD-25 in two patients who had definite and marked disease of the liver than in patients without liver disease. One of his patients who had had hepatitis a few weeks before taking the LSD-25 showed only a very mild reaction to the drug.

We still have too few cases of this kind reported to permit any generalizations about the contribution of previous patterns of behavior to prolonged reactions. However, in Case No. 1, a lifelong reaction pattern of inward withdrawal during stress, a current stressful life situation and the mescaline seem to have combined in producing the prolonged reaction. This subject had from early childhood a tendency to withdraw into an inner world of fantasy during periods of stress. He had exhibited this in rebellious periods with his mother and had shown some of it with his wife. He took the mescaline during a time of stress shortly after his wife announced evidence of a pregnancy which she wanted but which he did not. The mescaline apparently augmented ordinary feelings of unreality and apathy to a degree thath became symptomatic for him. Then followed secondary anxiety and hypochondriacal delusions.

It is noteworthy that a psychotherapeutic approach to this subject's disorder with an interpretation of his symptoms from a motivational point of view seemed helpful, although it is not clear whether the improvement observed was due to the accuracy of the interpretation offered or to other aspects of the patient's relationship to the therapist-investigator.

Several factors might have accounted for the fact that this subject had no prolonged psychotic reaction after the first mescaline experiment. First, at that time, his wife had not announced her pregnancy; secondly, the patient on the first occasion was given sodium succinate intravenously which has been shown to act as a moderately effective antidote; and thirdly, the patient was obliged to work extremely hard on the day after the second mescaline experiment so that the effects of fatigue may have summated with those of the mescaline and thus promoted the continuation of the psychotic condition.

Investigators and therapists using these drugs naturally wish to prevent prolonged reactions to them and wish to eliminate subjects they believe disposed to such prolonged reactions. The two subjects reported here did have severe reactions at the height of the intoxication. Other subjects of our series, however, who showed reactions we judged equally severe did not have prolongations of the effects of intoxication beyond the usual period of 8 to 12 hours. The reports in the literature of other prolonged reactions either give no data bearing on this point or fail to show correlations between severity of reaction at the height of intoxication and prolonged effects. MORSELLI (1936) had prolonged effects and an extremely marked reaction at the height of intoxication, but he ingested almost double the usual dose of mescaline. Perhaps further studies will show in larger series that prolonged reactions occur chiefly in those subjects having severe reactions. As severe reactions seem to

occur chiefly in those with marked anxiety and inadequate defenses against its disorganizing effects, improved testing and interviewing procedures may permit the detection of those disposed to severe reactions in advance. Although both the subjects reported here (especially Subject No. 1) showed deviant responses in psychological test material compared to other subjects of the series, we shall not claim that we would have eliminated these subjects from the experiments if we had studied the test data before the experiments.

The subjective discomfort and potential dangers of prolonged reactions to mescaline and kindred drugs should encourage us to improve our methods of detecting and eliminating from experiments subjects who may be suspected of susceptibility to these reactions.

Summary

1. Two cases of prolonged reactions to mescaline (400 mg. orally, one lasting four days, one lasting eleven days are reported.

2. The two subjects showing these prolonged reactions had reactions during the periods of maximal intoxication which were more severe than those of most other subjects in a series of 47 experiments on 25 subjects. Their reactions, however, were not more severe than those of some other subjects who did not have prolonged reactions.

3. In both subjects the reactions resembled mild schizophrenic reactions. One subject had a life-long pattern of withdrawal into fantasies during stress. He took mescaline at a time of stress in his life. The mescaline seems to have reinforced his pattern of withdrawal making it temporarily of psychotic proportions.

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Brain Stimulation Reward "Thresholds" Self-Determined in Rat

By

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With 3 Figures in the Text

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Behavioral training procedures that permit an animal to stimulate his brain electrically through permanently implanted electrodes can provide unique information about central nervous system processes (OLDS, BRADY). These selfstimulation procedures may be extended quantitatively by allowing the magnitude as well as the rate of the stimulation to be manipulated by the animal; in this way, information about stimulation preferences and thresholds may be obtained and stable baselines established for the study of variables. In a recent paper (STEIN and RAY), we reported a technique for determining the preferred (selected as optimal by the animal) current intensity for stimulating positively reinforcing brain sites. In this report, we describe a related method which gives a continuous assay of the smallest current levels that will stimulate positive cell groups sufficiently to produce a rewarding effect.

Methods

The animals were trained in a small sound-resistant box with two levers in one wall. Responses at one lever (stimulation lever) delivered a brief train of positively reinforcing brain stimulation. Each succeeding response at the stimulation lever produced brain-shock rewards that decreased stepwise in intensity. In the present experiments, there were 15 equal current steps between a moderately reinforcing, but a clearly suprathreshold, top value (generally less than 10 ma) and zero ma. The current steps were programmed with a stepping switch, activated by operations of the stimulation lever, which introduced graded resistances into the stimulating circuit.

The second lever (reset lever) could be operated at any time to reset the current to the top step. The reset lever never gave brain shocks.

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Ideally, the method has the animal work the stimulation lever repetitively for the brainshock rewards until the current intensity is driven down below a maintaining or reinforcing level, respond once at the reset lever to reset the current to the top step, and then return to the stimulation lever and start the next cycle. This procedure may be recognized as an adaptation for animal subjects of the classical psychophysical method of minimal changes (GUILFORD) with an operant response (reset) substituted for a verbal judgment.

Continuous records of the current step at which resets occurred were drawn by a recording potentiometer whose input voltage, controlled by the stepping switch through an auxiliary bank of resistances, was always proportional to the stimulating current. A cumulative response recorder and impulse counters simultaneously provided records of the self-stimulation rate.

The stimulating waveform was supplied from a Lilly-type pulse-pair generator and is described in more detail elsewhere (LILLY et al., STEIN and RAY). The paired pulses were opposite in sign, each 50 μsec in duration, and separated by 200 μsec. A brain-shock reward in the experiments reported here consisted of a 0.25 sec current train at 50 pulse pairs/sec.

Six male albino rats with bipolar electrodes (0.01 inch platinum wires, twisted together and insulated except at the tips) permanently implanted in posterior hypothalamic and midbrain tegmentum sites have been studied. In preliminary training the animals learned to obtain fixed, moderately reinforcing (approximately 5 ma) brain shocks by operating first either lever and then only the stimulation lever. After the self-stimulation rates had stabilized, threshold-determination training was begun.

With assistance from the experimenter, the rats could freguently be trained in the essentials of this procedure in less than 20 minutes. This aid consisted mainly in resetting the current to the top step when it was driven to zero or near-zero levels by the animal. Training was greatly facilitated by "shaping" the reset response; this was accomplished by resetting the current at first only when the animal oriented toward the reset lever, and then later requiring approaching and investigating responses at this lever before resetting.

Results

After a few two or three hour sessions, stable cycles of selfstimulation and resetting as described above were obtained with most animals. Current resetting was quite precise; even with small intensity steps (roughly, 1—2 per cent of the total range that gives reinforcement), a good animal would reset at the same step 50—60% of the time, and place 90—95% of the resets within one step above and below the modal

step. Typically, the threshold would increase during the first 10—20 minutes of an experimental session; then it leveled off and was stable for hours, although variability increased somewhat as the session progressed. With rest periods of 2—3 days between experimental sessions, performance has been found, at least for the two animals most extensively studied, to be stable for more than three months.

A representative record of threshold-determining behavior obtained from a well-trained animal with an electrode in the midbrain tegmentum is shown in the right-hand portion of Fig. 1. The recording pen moves downward one step (0.42 ma) with each successive response at the

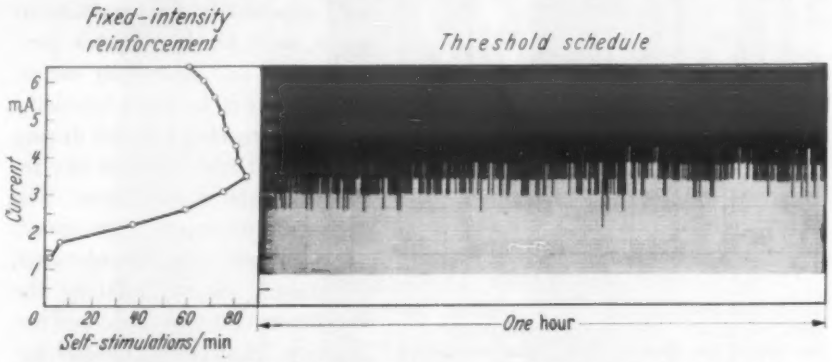

Fig. 1. Comparison of two indices of the threshold for brain-stimulation reward: (left) self-stimulation rate obtained with fixed-intensity reinforcement vs. (right) current intensity at reset in threshold schedule. The horizontal markings to the left of the threshold record indicate the 16 current levels (15 steps) available from 6.4 ma to zero ma. In the one-hour period shown, the animal gave himself 4,247 brain shocks and reset the current 558 times

stimulation lever, and returns to the top (6.4 ma) with every response at the reset lever; hence, the jagged edge of the curve indicates the current intensities at which resets occurred. On the left, plotted against the same current scale, are the self-stimulation rates obtained when the current steps were presented singly, each for several minutes, as fixed-intensity reinforcements for lever-pressing. In this manner, estimations of the "reinforcement threshold" given by the present technique may be directly compared with the minimum current level required to maintain self-stimulation in the fixed-intensity situation.

Fig. 1 shows that resetting tended to center about an intensity level (3.4 ma) that maintained a high self-stimulation rate, but below which performance dropped off sharply[1]. Similar findings were obtained from

[1] Studies of intensity preference indicate that the fall-off in selfstimulation rate with intensities higher than 3.4 ma should not be attributed to a decline in reinforcing value. For the preparation shown in Fig. 1, the preferred intensity is about 25 ma, and occasionally, after long periods of stimulation, values as high as 50 ma may be selected. Possibly, this decline in rate reflects longer lasting aftereffects of higher intensity brain shocks; put loosely, the rate declines because the animal does not "need" the next stimulation as soon.

a second tegmental animal and an animal with an electrode in posterior hypothalamus. The new procedure thus seems to give, at least for these electrode placements, an estimate of the reward threshold that is slightly higher than the minimum maintaining level for self-stimulation under fixed-intensity reinforcement[1].

The effect of manipulating the frequency (repetition-rate) of the stimulating waveform is depicted in Fig. 2. Two similar experiments are shown, one involving relatively coarse intensity steps (0.75 ma) and the other finer steps (0.5 ma). In both experiments the animal was exposed to five repetition-rates, each for 15 minutes, presented in an ascending series. Each point is the mean intensity at which resets occurred during the last 10 minutes that any repetition-rate was in force. The experiments, in good agreement, gave negatively accelerated, decreasing curves relating the reinforcement threshold and frequency. That the technique discriminates so clearly a shift from 25 to 33 cps testifies to its sensitivity.

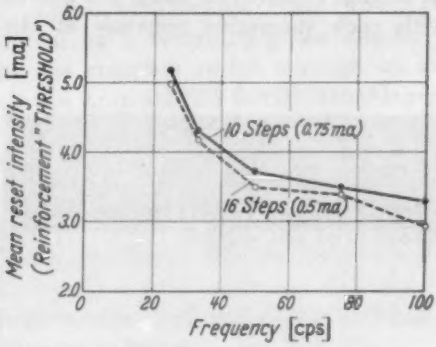

Fig. 2. Rat H-38 Posterior Hypothalamus. Reward "threshold" (mean intensity at reset) as a function of the repetition-rate of the stimulating waveform. Each point is the average current intensity of approximately 75 resets obtained in 10-min test periods

Some sample drug actions are shown in Fig. 3. Chlorpromazine hydrochloride at 1.5 mg/kg raised the threshold for reinforcement (resets at higher intensities) with the onset of action occurring about 15 minutes after the intraperitoneal injection. In contrast, *dl*-amphetamine sulphate (0.75 mg/kg) lowered the threshold (resets at smaller intensities) with the first indications of activity at about 5 minutes[2]. A more complete report describing the actions of other centrally-acting drugs [e. g., reserpine, phenethylamine, sodium pentobarbital, serotonin, LSD-25, phenylisopropylhydrazine (JB-516)] is in preparation.

[1] It will be recalled that the range of current intensities used for the threshold determination occupies only a small segment at the low end of the total range giving reinforcement (footnote 1, p. 253). The discrepancy, then, between the estimates of the "reinforcement threshold" given by the two procedures, although reliable, is relatively minor.

[2] The rate of self-stimulation was increased by this dose of chlorpromazine and decreased by amphetamine. These effects on rate may appear at first to conflict with the threshold data, but, following the reasoning in footnote 1, p. 253, these actions may be reconciled by assuming that chlorpromazine shortens and amphetamine prolongs the aftereffects of a brain shock.

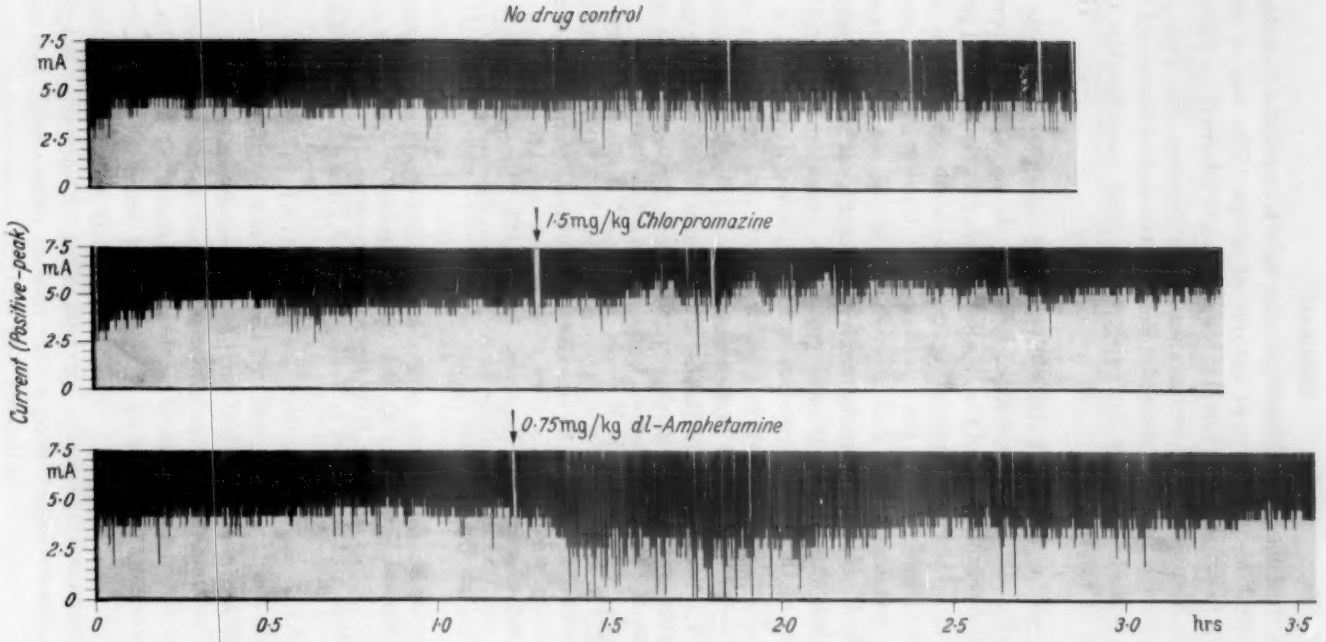

Fig. 3. Some sample drug actions mapped through changes in the "threshold" for brain-stimulation reinforcement. Each record shows the course of reset intensities for an entire daily session. The ordinal markings indicate the 16 current levels that were available. Rat H 38 Posterior Hypothalamus.

Comment

The method has considerable promise as a tool for the investigation of the central nervous system actions of drugs. The use of discrete stimulation to specific brain structures gives some information about sites of action. Because the determination is continuous, the time course of drug action is directly charted. Finally, the use of threshold intensities for stimulation may be expected to lend considerably to the sensitivity of the method.

Summary

The self-stimulation technique of OLDS is modified to permit a continuous determination of the smallest current levels that will produce a rewarding effect by stimulating positive brain sites. The animal receives brief brain shocks that decrease in intensity in small steps by operating one lever, and indicates which current step stimulated insufficiently by resetting the current at a second lever. Preliminary data suggest that the technique will provide a valuable tool for the investigation of the central nervous system actions of drugs.

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Letters to the Editor · Briefe an die Herausgeber · Lettres à l'éditeur

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Clinica delle Malattie Nervose e Mentali della Università di Sassari/Italia
(Dir.: Prof. S. Rigotti)

Studies on Cross Tolerance with LSD-25, UML-491 and JB-336

By

Antonio Balestrieri

(Received August 24, 1959)

Recent researches raise the problem of wether cross tolerance among hallucinogen drugs depends on a similarity of pharmacological effect or on a similarity of chemical structure.

In our experiments (Balestrieri and Fontanari), BOL 148, given at increasing dosages (up to mg. 2 a day) for 4—8 days did not consistently antagonize the effects of LSD 25 (mcg. 160). However, a certain degree of tolerance to LSD 25, at lower dosages than ours (50 mcg. or mcg. 1/kg.), was obtained giving BOL 148 for 5—7 days by Abramson *et al.* (total dosage 0,85—1,45 mg.) and by Isbell *et al.* (3 mg. a day). A good resistance to LSD 25 (50—75 mcg.) was reported by Ginzel and Mayer Gross, after only a 1—2 day administration of a few mg. of BOL 148. With a very prolonged administration (4—5 weeks) of BOL 148 at high dosages (30 mg. a day) Turner *et al.* obtained a complete resistance to LSD 25 (150—200 mcg.). Isbell *et al.* ruled out competition between LSD 25 and BOL 148, while Murphree *et al.* reported that the latter drug antagonized the effect of the former on blood pressure and pupil size, but not on mental conditions. Abramson *et al.* showed that MLD 41, given for 6 days up to mcg. 350, protects man against 100 mcg. of LSD 25. Cross tolerance between LSD 25 and LAE 32 and BOL 148 was reported by Cholden *et al.*, but they did not observe the same phenomenon between LSD 25 and mescaline. Hosko and Tislow have not been able to induce a resistance to the blood pressure effects of mescaline in dogs treating them previously with LSD 25. Cross tolerance between LSD 25 and mescaline was observed in rats, by Freedman and Aghajanian.

Former experiments performed by ourselves (Balestrieri-Balestrieri and Fontanari) showed that a good degree of cross tolerance seems to exists between LSD 25 and mescaline.

We performed another group of experiments, testing tolerance to LSD 25 after a treatment with UML 491, and tolerance to JB 336 after a treatment with LSD 25. The trials, according to the method previously described (BALESTRIERI and FONTANARI), were carried out on 13 patients, not aware of the experimental design. The diagnosis was polyneuritis, psychoneurosis, multiple sclerosis, myopathia. Controls were possible in 9 of the same subjects and were made before the tolerance trials, or at least 6 days later. Drugs were given orally. In 5 patients, after a treatment with UML 491 for 5—6 days (2—4 mg. a day), the response to LSD 25 (mcg. 200) was very mild. The control with the same dose of LSD 25 was possible in three of the subjects, and it showed a much stronger reaction both from the psychic and the autonomic point of view. In 4 other subjects, a single UML 491 administration (3 mg.) one hour before LSD 25 (mcg. 150) seemed not to antagonize in any way the latter drug. Actually, we have been able to induce only one subject to repeat the trial with LSD 25 alone, at the same dosage, and the reaction was somewhat milder. A simple antagonism between the two drugs seems probably to havebeen ruled out. In 4 other patients who had acquired a good tolerance to LSD 25 after a prolonged administration of the drug (up to mcg. 200 daily, for 4—8 days) the effect of JB 336 (10 mg.) did not appear consistently different from that obtained in the control performed on each subject with the same dosage of the second drug. The difference was of the same order than in our trials with LSD 25 after a prolonged treatment with BOL 148 (the average decrease was 22% and 12%, respectively).

A few additional experiments were recently performed with LSD 25 and psilocybine. In two psychoneurotic patients, a tolerance to the latter drug (6 mg. intramuscularly) seemed not to appear after a treatment with the former (during 5—8 days, up to 200 mcg. orally). Both cases were controlled with the same dosage of psilocybine. A tolerance to psilocybine seems very difficult to be achieved even with repeated administration of the drug itself. This type of experiment was also performed in other three psychoneurotic patients, with a daily dosage of 3—6 mg. (orally or intramusculary) for 7—9 days.

On the basis of our present knowledge, cross tolerance among lysergic acid derivatives seems to occur also if the psychotomimetic activity (like with BOL 148 and UML 491) is low or almost absent at the given dosages. Our studies on mescaline suggest that cross tolerance can also depend on similarity of pharmacological effect. However, cross tolerance did not appear, at least with a comparable intensity, in our trials with LSD 25 and JB 336 or psilocybine.

Further controls are needed in order to clarify the problem.

d-Lysergic Acid Diethylamide (LSD 25), 1-Methyl-d-Lysergic Acid Butanolamide (UML 491) and O-Phosphoril-4- Hydroxy-N-Dimethyl Tryptamine (Psylocibine) were kindly supplied by Sandoz S.A., N-Methyl-3-Piperidyl Benzilate (JB 336) by Leo G. Abood Ph. D.

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Bibliographies of Current Literature · Literaturübersichten Bibliographies

Psychopharmacology — Problems in Evaluation. Edited by Jonathan O. Cole and Ralph W. Gerard. 662 pages. Publication 583 National Academy of Sciences — National Research Council. Washington 25 D. C. 1959.

An important conference on the evaluation of pharmacotherapy in mental illness was held in Washington in September 1956. It was sponsored by the National

Institute of Mental Health, The National Academy of Sciences — National Research Council and The American Psychiatric Association. A planning group had met some months before and had set up five committees: one of these was concerned exclusively with overall planning and co-ordination, another with the preliminary screening of drugs and the other three with the clinical problems of patient selection and controls, test conditions and evaluation respectively. Four of these committees invited consultants to join in the preparation of working papers which formed the basis of their discussions during the first two days of the conference. The results of these discussions were then summarised at a plenary session which was attended by nearly 1000 people.

This book contains the substance of the work which went into the several meetings. It includes the working papers presented to the planning committee; the working papers which were presented to the ad hoc committees and the subsequent discussions; the summaries presented to the main conference and an edited version of the discussions there. The editors have also included other material when this seemed relevant.

The results of these labours constitute an impressive demonstration of the American contribution to psychopharmacology. The distinguished representatives of the natural sciences, the social sciences and clinical psychiatry covered most of the field and the formal papers are of the standard which one would expect from the participants. If one singles out the KILLAMS' "Methodological Considerations in Neuropharmacological Research", KRAMER and GREENHOUSE on "Determination of Sample Size and Selection of Cases", WIKLER on "The Loci and Mechanisms of Action of Phenotropic Drugs Considered in Relation to Screening Procedures" and GREENBLATT and LEVINSON on "The Sociopsychological Aspects of the Evaluation of Therapeutic Change" it is to illustrate the scope of the material rather than to imply any differences in quality.

The sections devoted to discussion may present more difficulties to the reader, perhaps especially the European reader, who is less accustomed to this mode of presentation. Some of the discussions undoubtedly serve to convey vividly the clashes of opinion which attend some of the basic problems of psychopharmacological research. In this category, for example, would come the disagreement between Dr. KETY and Professor SKINNER (p. 231) on the value and limitations of operant conditioning techniques; similarly, Dr. FREYHAN's vigorous defence of the clinical standpoint (p. 333) provides a stimulating introduction to his paper on "Selection of Patients from the Clinical Point of View" (p. 372). Further, the discussions contain some statements like those of Dr. GRUNDFEST (p. 165) and Dr. BRAZIER (p. 187) which are brief communications in themselves. On the other hand, some passages are repetitious and confusing and the studious reader may well be distracted by the entertaining jokes and the more exuberant exchanges which lubricated the proceedings.

But these are minor strictures on a volume which stands out as a landmark in the literature of psychopharmacology and which carries the stamp of authority in so much of its content. As a work of reference and as a commentary the book remains indispensable to everyone interested in the theory and practice of psychopharmacology. And if, as Dr. FELIX points out in his introduction, the most important function of the conference was to effect "an exchange of ignorance" then we are now able to see that it also prepared the ground for a number of sites on which work has been in progress during the past four years.

MICHAEL SHEPHERD, D. M. (London)

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8. *Les références du texte* seront suivies du nom de ou des auteurs (éventuellement entre parenthèses) s'il n'y en a pas plus de deux. Dans les autres cas, le premier seul est cité suivi de «et al.» Si le même, ou les mêmes auteurs, sont plusieurs fois cités, on fera suivre le nom de la date de parution de chacune des œuvres nommées. Au cas où plusieurs ouvrages du ou des mêmes auteurs dateraient de la même année, on ajoutera à la date les lettres a, b, c, etc.

Les œuvres citées seront classées à la fin de l'article dans une bibliographie sans numération, par ordre alphabétique selon le nom du premier auteur, de la date de parution et des lettres a, b, c, etc. dans le cas ou il existerait plusieurs ouvrages du même auteur.

Les références des œuvres citées se présenteront comme suit: nom de l'auteur, initiales des prénoms, titre complet dans la langue originale, titre de la revue (abrégé selon le système des World Medical Periodicals, 2ème édition 1957, World Medical Association), tome, numéro de la première et de la dernière page, année (entre parenthèses).

Exemple: DEWS, P. B.: Studies on behavior, IV. Stimulant actions of methamphetamine. J. Pharmacol. exp. Ther. **122**, 137—147 (1958a).

Les livres seront cités précédés du nom et initiales des prénoms du ou des auteurs; titre complet, édition, lieu de publication, éditeur, date de parution.

Exemple: BLEULER, E.: Lehrbuch der Psychiatrie, 9. Aufl., Berlin-Göttingen-Heidelberg, Springer 1955.

9. Il sera fait de chacun des articles publiés 75 tirés à part mis à la disposition des auteurs à titre gratuit.

10. Les **manuscrits originaux accompagnés d'un double** doivent être envoyés
   a) pour les U.S.A. et le Canada au
      Dr. A. WIKLER, National Institute of Mental Health, Addiction Research Center, U.S. Public Health Service Hospital, P.O. Box 2000, Lexington, Kentucky
   b) pour les autres pays au
      Prof. Dr. E. ROTHLIN, Sonnenweg 6, Bâle, (Suisse)
   c) ou à tout autre membre de la rédaction selon le choix de l'auteur.

11. **Les éditeurs et les rédacteurs prient instamment d'observer strictement ces directives aussi bien en ce qui concerne le caractère original du manuscrit que les détails techniques. La collaboration entre auteurs et éditeurs en sera ainsi facilitée et les delais de publication raccourcis.**

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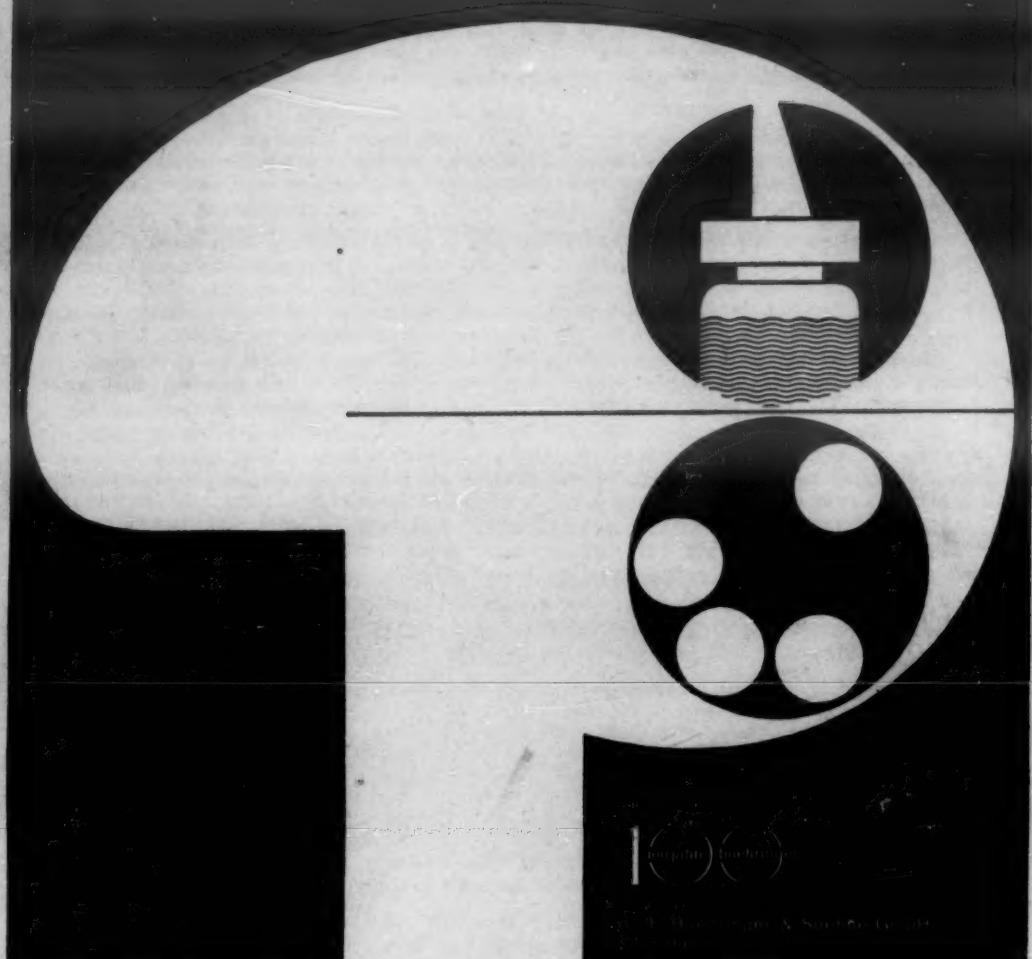

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